

Microbiology

STAINING

- GRAM STAINING
- Is a differential method of staining
- Other differential stains

↓

ZN STAIN (Ziehl Neelsen Stain)

- Gram Stain differentiates between → Gram positive and Gram negative
- Acid fast stain differentiates between → Acid fast / Non-acid fast

Sequence of Gram Staining

Mnemonic:

Methyl G A S

Methyl violet / Crystal violet / Gentian violet (Primary stain)

↓

Gram's Iodine (Mordant-fixer)

↓

Acetone / Acid alcohol / Ethyl alcohol (Decolorizer)

↓

Safranin (Counter-stain)

Gram positive bacteria → purple to violet

Gram negative bacteria → Pink

Gram positive bacteria → thick peptidoglycan layer in cell wall

↓

Does not allow primary stain to go off after adding decolorizer

↓

Retain primary stain, do not take up counter stain

↓

Look purple to violet

Gram negative bacteria. → Thin peptidoglycan layer in cell wall

↓

Lose primary stain after decolorizer

↓

Take up the counter stain (Safranin)

↓

Look Pink

2. ZN STAIN / ACID FAST STAIN

Sequence:-

Sputum Smear
↓
Carbol fuchsin
↓
Intermittent Heating (fixing)
↓
20% H_2SO_4 (decolorizer)
↓
Methylene blue (counterstain)

3. KINYOUN METHOD OF STAINING

Carbol fuchsin + phenol
↓
20% H_2SO_4
↓
Methylene blue

No Heating up – So, COLD method of staining

Examples of Acid fast

- 20% Acid fast → *Mycobacterium tuberculosis*
- 5% Acid fast → *M. Lepra*
- 1% Acid fast → *Nocardia*
- 0.25-5% Acid fast → Oocyst of – *cryptosporidium parvum*,
Cyclospora, *Isospora*
(These are strongly associated with HIV+VE)

→ All Cocci are mostly G+ve, except

- Meningococci
- OG-ve cocci
- Gonococci
- Veillonella
- Moraxella

→ All bacilli are G-ve, except

- M – *Mycobacterium* species
- A – *Anthraxis Bacillus*
- C – *Clostridium* species
- D – *Diphtheriae Corynebacterium*
- O – G+ve Bacilli
- N – *Nocardia* species
- A – *Actinomyces* species
- L – *Listeria monocytogenes*
- D – *Diphtheroids*

- Staining at poles better
- in between poorly stained
- (Like in a safety pin appearance)
- Seen in → *Yersinia pestis*
 - *Klebsiella granulomatis*
 - *Burkholderia mallei*
 - *Burkholderia pseudomallei*
 - *Vibrio Parahaemolyticus*
 - *Haemophilus ducreyi*

Capsulated organism:

- P → *Pneumococcus*, *Pseudomonas*
- A → *Anthraxis Bacillus*
- K → *Klebsiella Species*
- I → *Influenzae (Haemophilus)*
- Y → *Yersinia Species*
- B → *Bordetella Species*
- M → *Meningococci*
- C² → *Clostridium Perfringens (Welchii)*, *Cryptococcus Neoformans*
- V → *Vibrio parahaemolyticus* → Most Capsules are made up of Polysaccharide but exception:

Polypeptide capsule (*Bacillus Anthracis*)

- *Pneumococcus* shows – **Quellung reaction**

↓

Quellung reaction is Capsular Smelling

↓

Capsule Swells because of the serological reaction (Capsular Antigen with Capsular Antibody)

- Colony from culture of *Bacillus Anthracis*:

↓

Add Methylene Blue

↓

Capsule gets stained. This reaction is called as MCFadyean's reaction

Function of Capsule:-

- Avoids Phagocytosis by preventing the process of opsonization
- Helps in adherence to surfaces

Opsonisers: – C₃b (most important)

- Fc Segment of IgG
- IgM.

MOTILITY



Bacteria are motile by the presence of Flagella

Various Motility of organisms

- Tumbling Motility – Seen in listeria
- Darting Motility – Seen in vibrio
- Swarming – Seen in Proteus
- Falling leaf motility – Seen in Giardia Lamblia
- Corkscrew motility – Treponema Pallidum
- Lashing Motility – Seen in Borrelia

→ These Motilities are demonstrated by 2 Methods.

- Hanging Drop Method
- Usage of Semi-Solid agar (Motility agar)

→ Giardia Lamblia is having 4 pair of flagella

→ Spirochetes (eg. Treponema pallidum) have Endo flagella;
It arises from Periplasmic space

Culture Media

Simple Media

Nutrient	Nutrient
Agar	Broth (liquid)
(NA)	(NB)

(2% agar for Solidification) No agar

- Nutrition is provided by Peptone and Meat extract
- Agar is only a solidifying agent.

Complex media:

Enriched media



Always Solid

(Blood agar; Chocolate agar)

→ In Blood agar 5% Sheep Blood is used

→ In chocolate agar there are 2 factors

X (haemin) and **V (NAD)**

Enrichment media:

→ Always liquid

→ eg: Selenite-F. broth

Used for salmonella and Shigella

Selective Media:

→ Mostly solid

- L.J Media (Lowenstein Jensen Media)

↓

Used for M.T.B

Only MTB will Grow Not other will Grow.

→ This is made Selective by Malachite Green

→ TCBS

T – Thiosulphate

C – Citrate

B – Bile Salt

S – Sucrose

↓

TCBS is used for *Vibrio Cholerae*

→ Bile salt acts as a Selective agent.

Differential / Selective / Indicator

→ Mac-Conkey Agar

Content of Mac-Conkey agar

P – Peptone

L – Lactose

A – Agar

N – Neutral red (selective)

T – Taurocholate Sodium

→ It distinguish between lactose fermenter (LF) and non-lactose fermenter (NLF)

LF

NLF

- | | |
|----------------------|-------------------------|
| • Lactose fermenter | - Non lactose fermenter |
| • Pink Colour change | - No colour change |

→ CLED

- Cysteine Lactose Electrolyte Deficient media
- Indicator, differential media, non-selective; urine samples are used
- Better than Mac-Conkey Agar

→ Transport Media

- Maintains viability of organism but does not allow multiplication
- V-R media → Venkat Raman Rama Krishnan media for - *V. Cholera*
- Carry Blair → *V. cholerae*
- Pike's Media → *Streptococcus pyogenes*
- Sach's Buffered glycerol saline → *Shigella* + *salmonella*

Classification based on Oxygen Requirement

- Obligate aerobe – need O_2 desperately eg. *Pseudomonas*, MTB
- Obligate anaerobe – Do not need O_2
 - A – Actinomycetes
 - B – Bacteroides
 - C – Clostridium
- Facultative anaerobe – they are aerobes but can be anaerobe A/K/A changed Anaerobes.
 - Eg Staph / strepto
- Microaerophiles – Need 5% O_2 (less O_2) eg. campylobacter
- Capnophiles – CO_2 loving eg.
 - *Brucella abortus*
 - *Streptococcus Pneumoniae*
 - *Haemophilus influenzae*

Based on Temperature Requirement

Psychrophiles → $-10^{\circ}C$ – $+10^{\circ}C$

Or $-20^{\circ}C$ – $+20^{\circ}C$ (Some sources)

Mesophiles → $20^{\circ}C$ – 40°

(medically important)

Thermophile → $> 45^{\circ}C$ for some mins

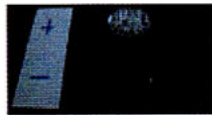
$> 55^{\circ}C$ for some mins

Can even live in $> 100^{\circ}C$

Eg. *Thermus Aquaticus* (used in PCR)

Bacteriology

Gram Positive Cocci (GPC)

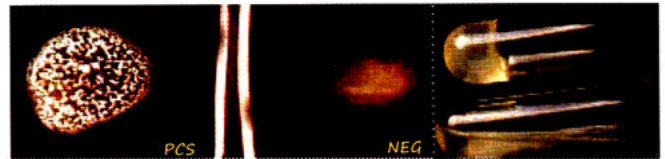


(Coagulase test)

Staph +

- Strepto

Catalase test

Coagulase +ve
Pathogenic staph

S. Aureus

Coagulase -ve
Non pathogenic staph

(CONS) (Coagulase Negative Staph)

Staph Epidermidis

Staph Saprophyticus

Sensitive -- Novobiocin Testing -- Resistant

Novobiocin testing done on → (MHA) → Mueller Hinton Agar Media

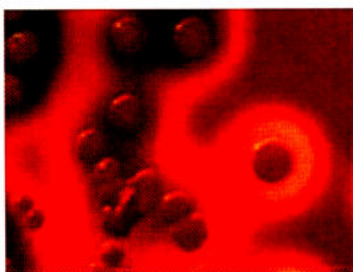
Method used is Kirby Bauer Disc Diffusion Testing (KBDDT)

CONS

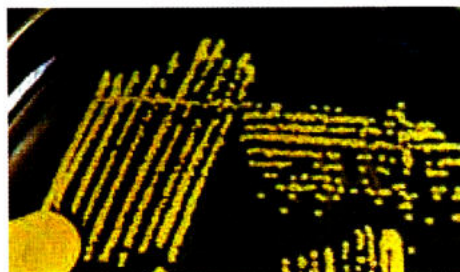
- S. Epidermidis → Intervention → i.v. cannula Catheter
→ Biofilm formation
- S. Saprophyticus → A young female develops UTI
MC GPC – Staph saprophyticus

Staphylococcus Aureus

- GPC; catalase +ve; coagulase +ve
- Violet Grape like clusters
- Pin head size of colonies & hemolysis on blood agar
- Golden yellow pigment on nutrient agar
- Most important / Best selective media is Mannitol salt agar (Ludlam's media)



Beta hemolysis

Golden yellow pigment
on nutrient agarMannitol salt agar
(Ludlam's media)

Virulence Factors

Protein A → Binds Fc portion of IgG

→ Cowan - 1 strain of *S. aureus* has Protein A



It is responsible for coagglutination reaction.

Hemolysins → α Hemolysin and β - Hemolysin

→ α Hemolysin - Shows paradoxical action → inactivates at 70°, reactivates at 100°C

→ β Hemolysin → Shows hot & cold phenomena

→ Starts at 37°C

→ Best seen at 4°C (Cold)

→ Leucocidin (Panton Valentine toxin) → Kills leucocytes

Toxin Mediated Illness

(1) Food poisoning- *S. Aureus* → Enterotoxin A (MC) is responsible

→ Pre formed Toxin

→ Because of contaminated meat & milk

→ Heat stable toxin

→ Vagomimetic action

Mimics *b. Cereus*

→ Emetic strain

→ Same ICP - 1-6 hours (Incubation period)

→ Reheated Chinese fried rice

→ (2) S S S S (Staphylococcal Skin Scalded Syndrome)

→ ET- A

Exfoliative toxin or entero toxin

→ ET-B

Termed as

TEN In Adult (Toxin Epidermolytic Necrosis)

Ritter's disease - In Children

→ May act as super antigen

→ (3) TSST (Toxic shock syndrome toxin)

→ Enterotoxin F (Super Antigen)

→ Shock like features

→ Associated with use of contaminated vaginal tampons

Disease 1. Carbuncles

2. Furuncles

3. Cellulitis

4. Impetigo

5. Botryomycosis (Mycetoma like lesion)

6. Tropical Pyomyositis

7. Acute Osteomyelitis

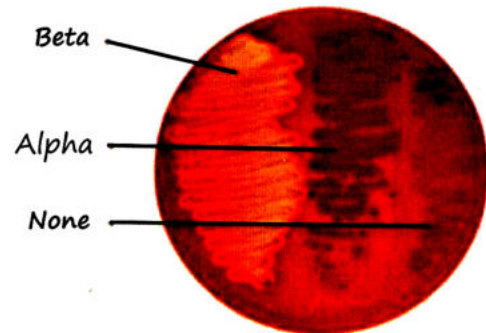
8. Native valve endocarditis

→ IV drug abusers → Rt sided valve endocarditis.

9. Pneumatocoles

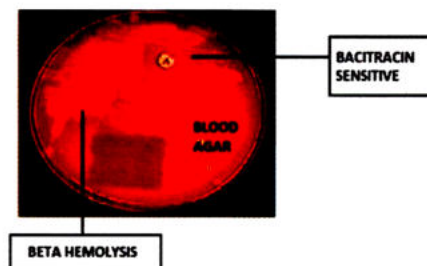
MRSA

- Main mechanism → *mecA* gene acquired
- PBP (Penicillin Binding Protein) → PBP 2a
- Extra chromosomal → Production of lactamases
- Cefoxitin screening Test is better than Oxacillin screening test
(Cefoxitin) Resistance → 100% MRSA
- PCR for *mecA* gene – Best for Dx
- MC mechanism for resistance transfer in *S. Aureus* is transduction. (Bacteriophage mediated)
- Phage Typing
- MC – 80/81 A → Nosocomial epidermics
- 23 types of phages for staph aureus
- DOC for MRSA → Vancomycin

**Streptococcus****Classification**

α Hemolysis	β Hemolysis	γ Hemolysis
<i>S. Pneumococcus</i>	Classified into A–V based on Carbohydrate 'C' in the cell wall.	Enterococcus
<i>S. Viridians</i>	A is divided into 8 types based on M protein (Griffith typing)	
	Group A. <i>S. pyogenes</i> B. <i>S. agalactiae</i> C. Enterococcus	

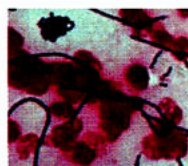
- Mnemonic**
- Lucknow City Good Morning
 - Lancefield → Carbohydrate
 - Griffith → 'M' Protein

Hemolytic Streptococci**To Differentiate**

	<i>S. Pyogenes</i>	<i>S. Agalactiae</i>
Bactracin Test	Sensitive	Resistant
CAMP Test	⊖	⊕
Hippurate Hydrolysis Test	⊖	⊕

S. Pyogenes

- GPC, Chains (Violet)



Culture Media

→ CVBA (Crystal violet blood agar)

Pin Point Colonies

Transport Media → Pike's Media

Diseases

1. Acute pharyngitis
2. Necrotising fasciitis (Flesh eating bacteria)
3. Cellulitis / impetigo
4. Scarlet fever toxin / Erythrogenic toxin A & C (Super antigen)

Non Suppurative Complications

ARF (Acute Rheumatic Fever)

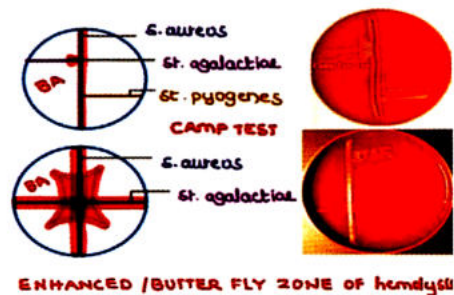
- H/o sore throat
- Lifelong prophylaxis of penicillin
- ASO titres > 200 Todd units

AGN (Acute Glomerulonephritis)

- H/o pyoderms infections
- Spontaneous remission
- Anti DNA are B>300-350 Units Anti-hyaluronidase

Group B Streptococci / *S. Agalactiae*

- Early & late neonatal meningitis
- CAMP Test → - + ve (i.e. enhancement of β hemolysis between *S. Agalactiae* and *S. Aureus*) (Christie Atkins Munch Peterson)
- Bacitracin resistant
- HHT (Hippurate hydrolysis Test) - + ve



Viridans

S. Viridans

- ↓
- SABC (Subacute Bacterial Endocarditis)
- Late onset prosthetic valve endocarditis (>1yr)
- 1. Early prosthetic valve endocarditis → *S. Epidermidis* (1yr)
- 2. Native valve → *S. Aureus*
- 3. IV drug RSVE → *S. Aureus*
- 4. IV drug LSVE → *Enterococcus*

S. Mutans

- ↓
- Dental caries

Enterococcus

- *E. Faecalis* / *Feacium*
- Normal commensal of GUT
- Culture media → Bile desculin agar (Black colored colonies)
- DOC → 1. Penicillin + Amminoglycoside
- 2. Vancomycin. Resistant Enterococcus (VRE)
- Because of mutation of van gene
- Changes in cell wall structure includes

D alanine D Alanine into → D alanine D lactate
Or

→ D alanine D serine variations

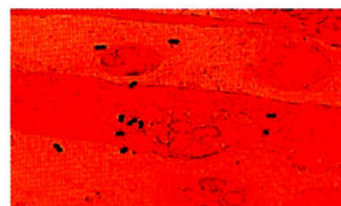
DOC for VRE → Linezolid / Daptomycin

Enterococcus causes

- Surgical wound infections
- In IV drug abusers – LVSE (Left Sided Valve Endocarditis)

S. Pneumoniae / Pneumococcus

- GPC
- Capsulated (Quellung's reaction) → Capsular swelling due to serological reaction
- Lanceolate shaped (Flame shaped)
- Diplococci
- Draughtsman like appearance on (Carom coin appearance) Blood agar & chocolate agar



Diplococci

Biochemical Reactions

- +ve {
- B** → Bile soluble
 - I** → Insulin fermenter
 - O** → Optochin Sensitivity



Optochin sensitivity

Disease Caused

- C** → Conjunctivitis
- O** → Otitis media (MC)
- M** → Meningitis (M/C cause in adults)
- P** → Pneumonia (Most virulent in Type 3)
- S** → Sinusitis

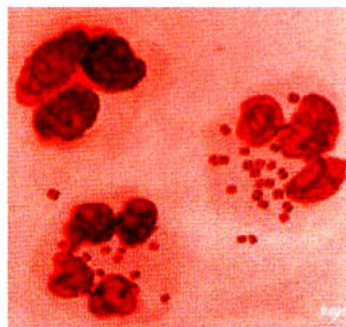
Vaccines

- 23 valent → to be given to splenectomised individuals
- 13, 14 valent
- 7 valent
- CSF shunt surgery
- Cochlear implants
- DOC → Penicillin
- Mechanism of Resistance is PBP changes to PBP 2b.

Gram Negative Cocci

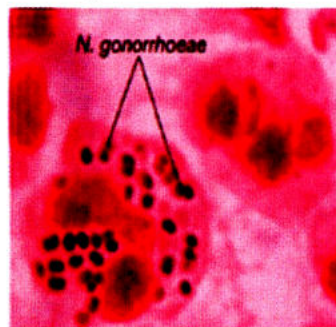
Meningococci

- Capsulated
- Lens shaped
- Ferments glucose & maltose
- Mainly intra/ extra cellular both



Gonococci

- Non capsulated
- Kidney shaped
- Ferments only glucose
- Mainly intra cellular



Meningococci

- On basis of capsular polysaccharide it is being classified into groups A, B, C.
- Source → Normal Nasopharyngeal Carriers (5 -15%) and cause (Droplet spread) → (Nasopharynx)
- Meningococcal meningitis
- Fever, headache, vomiting, neck rigidity, rash
- Lab diagnosis
- 1. Blood culture / CSF culture
 - Culture is being done on Thayer Martin media, Mueller Hinton agar
 - MHA (Mueller Hinton agar) is being used for Antibiotic sensitivity testing by Kirby bauer disc diffusion testing.
- MC dreadful complication → Water House Friderichsen syndrome → B/L adrenal hemorrhagic necrosis.
- Deficiency of → C_5 -C: MAC (Late complements / Membrane attack complex)
 - ↓
 - Prove for neisserial infections.
- MCV - 4 (A, C, Y, W)
 - (Meningococcal conjugate vaccine)
 - Group 'B' - no good vaccine is available.
- DOC for carriers → Ciprofloxacin
- DOC for cases → Ceftriaxone

Gonococci

- Pili/Auxotyped
 - ↓
 - Nutrient based
 - ICP → 2-7 days
- Gonorrhea → STD
 - Males → Urethritis
 - Females → Cervicitis
 - Newborn → Ophthalmia neonatorum
- Lab Diagnosis → Eye swabs
 - Endocervical swabs
 - Urethral swabs

Gram staining →

Culture media

↓

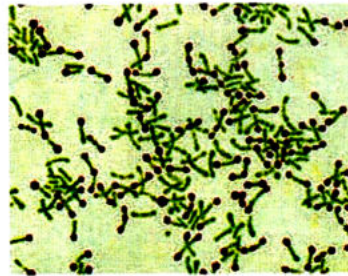
(Thayer Martin media)
(Modified New York agar)

Complications

- Watercan perineum (Mostly in Males)
- Fitz hugh Curtis syndrome
- Poly arthritis
 - ↓
 - Suppurative
- DOC → Ceftriaxone (Plasmid recruitment) + Resistance is ↑ing
- DOC for carriers → Ciprofloxacin → Rifampicin

Gram Positive Bacilli**Diphtheria (Klebs Loeffler's Bacilli)**

- Chinese letter pattern
- Granules → Metachromatic
 - Polymetaphosphate
 - Volutin
 - Babes Ernst granules



Chinese Letter Pattern

**Staining**

- P** → Ponder's
- A** → Albert's Stains it green
- N** → Neisser's

Cuneiform arrangement / Chinese letter pattern is seen

Contents of Albert Stain

- Toluidine blue
- Malachite green
- Glacial acetic acid
- KI + I₂

3 Biotypes

Gravis		Intermedius	Mitis
Colonies Look On potassium tellurite agar	Daisy head	Frog egg	Poached

- Diphtheria is toraemia not a bacteraemia
- Pseudomembrane formation seen → Greyish site → MC site → Faucial (Tonsillar)
- Bull's neck (Neck edema)
- Chronic complications
- Myocarditis
- Polyneuropathies

LD → 1. Toxin demonstration

- Elek get Precipitation Test
- PCR for tox gene
- ELISA for toxin production

Dx → 1. LSS (Löffler's serum slope)

Culture media (Colonies in 6-8 hours)

(Enriched media) – (Inspirated) used for early diagnosis of diphtheria.

2. Potassium tellurite agar → Best selective media.
3. Macleod's media
 - Diphtheria toxin
 - (Exotoxin – Needs Fe for action)



There are 2
components in toxin



Corynebacterium
diphtheria



Acts on elongation factor
(EF -2) and cause its ADP
ribosylation

SCHICK TEST → Neutralisation Test

Done for susceptibility of an individual to a bacteria

ST → +ve → needs immunization

ST → -ve → doesn't need immunization (Already immunized)

T/t → ADS (Anti diphtheria serum)

80,000- 1,20,000 units

→ Antibiotics

Non *Corynediphtheriae*

C. Minutissimum



Erythrasma (Coral red Fluorescence)

C. Parvum – Immuno modulator

Every CD is not pathogenic but is made pathogenic by phage (Tox gene) → Lysogenic conversion.

Gram Positive Bacilli

B. Anthracis

→ Box car appearance

→ Non – Motile; Capsulated (Polypeptide)

→ Spores → Bioterrorism (Category A bioterrorist weapon)

Appearances

M → Medusa head appearance

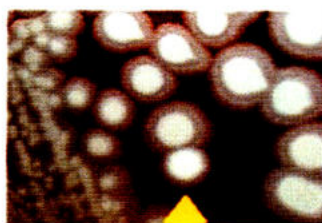
I → Inverted fir tree appearance / inverted Christmas tree / Gelatin Stab Culture)

S → String of pearl appearance (On penicillin agar)

Bamboo → Bamboo stick appearance / Box car appearance. (On Gram Staining)



Bamboo Stick Appearance



medusa head colonies
on blood agar

Mc Fadyean's Reaction → For Capsule Blue (Polypeptide)

→ Ascoli's thermo precipitation test → For anthrax antigen

→ Culture media → PLET

Diseases

1. Cutaneous anthrax (Hide porter's disease)

↓
Black eschar

↓
Malignant pustule (Edema)

→ Seen in veterinarian, Butcher, Porter

2. Pulmonary anthrax – wool sorter disease

To prevent it – Duckering (Pretreat the wool with formaldehyde to kill the spores) Intestinal Anthrax

By eating contaminated wild animal meat ingestion

→ Anthrax toxin mnemonic (England Premiere League)

E → Edema factor

P → Protected factor

L → Lethal factor

} synergy + ↑ cAMP

B. Cereus

Emetic

IP – 1-6 hrs

↓

Resembles the heat stable

Toxin of E. coli in action

↓

↑ cGMP

→ Chinese fried rice → Milk & meat ingestion

Culture media → B. cereus – MYPA

diarrhoeal

8-16 hours

↓

Diarrhoeal toxin

↓

↑ cAMP

Clostridium

→ Obligate anaerobe

→ Spore bearing

→ NC; Motile (Stately) exception is Cl. Perfringens
(Capsulated, non motile)

→ Terminal end round (Drum stick appearance)

→ Central support end sub terminal spore (Most of the clostridium)

Method of Anaerobiosis

→ Gas Pak – chemical anaerobiosis (M/C Done)

→ Culture Media

→ Robertson cooked meat media (RCM)

If color of meat pieces turns black then the organism is proteolytic (Cl. Tetani)

If pink then it is saccharolytic (Cl. Perfringens)

Cl. Perfringens

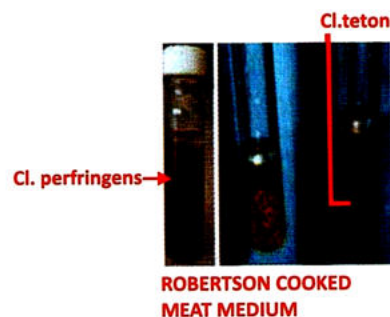
(Type A; Toxin) – Most infections

→ Causes gas gangrene (Clostridial myonecrosis)

ICP → 1-2 days

→ C/F pain + crepitus

→ Causative organisms



ROBERTSON COOKED MEAT MEDIUM

Cl **P** → Perfringens
N → Novyii
S → Septicum



Boat shaped bodies (Citron bodies)

- 2. Food poisoning – Heat labile enterotoxin
 ICP → (8-24 hours)
 Contaminated meat ingestion)

Type C, β – Toxin

Causes

Pigbel – Necrotising enteritis – Because of consumption uncooked pork

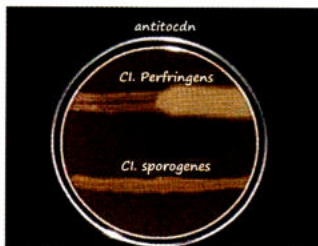
Reactions

Senior → Stormy fermentation of litmus milk

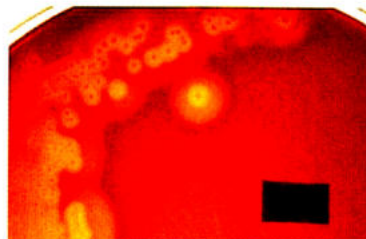
N → Nagler's Reaction (Neutralisation test) – Due to lecithinase (Toxin)

T → Target hemolysis (Double zone of hemolysis)

R → Reverse CAMP Test



Nagler's Reaction



Double Zone of Hemolysis



Stormy Fermentation



Cl. Tetani

Tetanus – 8th day disease

ICP → 8-10 days

ICP → <6 – 7 days ~ poor / worse prognosis

Tetanospasmin → Pre synaptic inhibition of glycine & GABA



Constant excitation



Lock Jaw

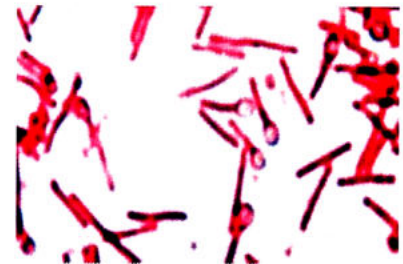
(↑ DTR)



Opisthotonus posture (bow like)

→ India is free of Neonatal tetanus

LD → Culture / PCR



Cl. Botulinum

Neurotoxin

(Most Potent toxin on earth)

→ Canned food poisoning (Diplopia, dysphagia, dysarthria)

Enterotoxin (C)



- ↓ DTR
- Constipation
- Infant botulism (Sporulated honey ingestion)
Flaccid paralysis (Floppy infant syndrome)
- Toxins have cosmetic purpose to remove wrinkles.
- Rx → Botox Treatment
- Used in eye surgeries such as blepharospasm strabismus.

Cl. Difficile

(Clostridium Difficile Associated Diarrhea) → CDAD

- Clindamycin (Quinolones, penicillin) (Broad spectrum antibiotics are causative agents)

Can
Lead
to

Pseudomembranous colitis

DOC → Oral Vancomycin

Metrogyl

Diagnosis

- Toxin demonstration (Since difficult to culture)
(Toxin A & Toxin B)
↓ ↓
Enterotoxin Cytotoxin
↓
(Demonstrated in stool on Hep2 cell lines)
- PCR for tox gene
- ELISA for toxin production
- Sigmoidoscopy – to visualise the ulcers

Mycobacterium TB

- 20% acid fast (Because of mycolic acid content)

Virulence Factors

- Cord factor
Inhibit phagolysosome fusion
- Lipoarabinomannan
- Heat shock protein (HSP)

- LD → 2 sputum samples
- Early morning sample
 - On spot sample

Petroff's Method (Liquefy The Sputum)

Acid Fast Staining / ZN Staining

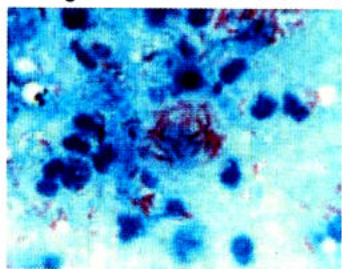
- ↓
Carbol fuchsin
↓ Intermittent heating
20% H₂O₄
↓

Culture on LJ media (Green)

- ↓
- Inspiosated
 - Rough
 - Tough
 - Buff colonies

Methylene blue

↓
→ blue back ground & Acid fast bacilli



→ Eugonic (Luxuriant Growth) (6-8 weeks)



Kinyoun Staining – cold method (Modification of Acid fast staining)

More concentration of phenol

↓

Fixing

→ Bactec / Bactalert – using liquid culture media (Middle Brooke's Media) → Early Diagnosis (Not Confirmatory)

→ MTB PCR

→ Gene Xpert Rif (Rifampicin resistance detected)

→ CBNAAT (Cartridge Based Nucleic Acid Amplification Test)

→ Live probe assays – multidrug resistance can be detected

Results are obtained < 2 hours

IGRA → Interferon gamma release assay

↓

Sensitized 'T' cell Activity Measured from blood aka quantiferon TB gold assay.

→ Antigens used are ESAT -6, CFP 10, TB 7.7

If sample load is high, then we go for fluorescent staining called Auramine Rhodamine.

False ve → high

Tuberculin Test / Mantoux Reaction

- 0.1 mL PPD
- Flexor aspect of fore arm
- After 3 days, calculate Induration diameter hardness
 - < 5 mm → -ve
 - 5 - 10 mm → Equivocal
 - > 10 mm → + ve

False +Ve Tuberculin Test Seen In

- Atypical mycobacterium infection
- Recent BCG vaccination

False - Ve (Cell Mediated Immunity ↓↓)

- Miliary TB
- Advanced stage of AIDS
- Malignancy

BCG

- Derived from Danish 1331 strain of Mycobacterium bovis
- ID route

- Diluent → Normal Saline
- Efficacy → 0-80%
- TB is a notifiable disease.

Atypical Mycobacterium

Runyon Classification

I. Photochromogens (Light)

- Myco - **M** → *M. Marinum*
A → *M. Asiaticum*
S → *M. Simiae*
K → *M. kansasii*

II. Scotochromogen (Darkness)

- S** → *M. Szulgai*
S → *M. Scrofulaceum*
G → *M. Gordonae*

III. Non Chromogens

- MAC (*Mycobacterium Avium Complex*) / Battey's bacillus
- *M. Xenopi*
- *M. Ulcerans* → Buruli ulcer

IV Rapid Growers

- *M. Chelonae* Associated with Post - Injection abscess → *M. Fortuitum*
- *M. Abscessus*
- *M. Phlei*
- *M. Smegmatis*

Swimming Pool Granuloma / Fish Tank Granuloma → *M. Marinum*

Buruli Ulcer

- *M. Ulcerans*
- Atypical Mycobacterium ATM mimics MTb in presentation → *M. Kansasii*

M. Leprae

- K/a Hansen's disease
- Doesn't follow Koch's postulates.
- Grows on Foot pad of Banded armadillo/ foot pad of mice



Ridley Jopling Classification

TT	BT	BB	BL	LL
↓	Most common in India			
Paucibacillary		Multibacillary		
Granuloma formation		Granuloma is absent in BL & LL		
Early involvement of nerve		Late involvement of nerve		

Lepromin Test

Early	Late
Fernandez	Mitsuda
> 3 days	> 3 weeks
Indurations diameter is measured	Nodule diameter measured

Lepromin test is a prognostic test not a diagnostic test.

Type I Lepra Reaction (Down Grading)	Type II Lepra Run (ENL) Erythema Nodosum Leprosum
→ Type IV hypersensitivity → MC nerve → ulnar → M/C Feature → edema	→ Type III HS → Because of TNF α release → Crops of nodules over skin

Both type I & Type II Lepra Reaction are emergency

DOC ~ Gluco – Corticoids	In type 2 Thalidomide can also be used.
--------------------------	---

LD (Lab Diagnosis) – Slit skin smear/ Nasal mucosal swabs

↓

Acid fast staining

5% H₂O₂ Used for decolorization

Bacteriological Index

< 2 = pauci

≥ 2 = Multi

T/t

Dapsone +

Clofazimine +

Rifampicin

→ Pauci

→ 6 months

→ Multibacillary

→ 12 months

Nocardia	Actinomyces
Strict Aerobes	Anaerobe
Pulmonary nocardiosis	Oral Actinomycosis
	MC site is cervico facial
	Sinus, swelling, sulphur granules.
	↓
	Crush them & see under microscope
	↓
	Sunray appearance.
N. cultured by paraffin bait technique.	Actinomyces israelii - PID in IUCD users.

Nocardia

Actinomyces

} Both together cause Actinomycetoma

Listeria Monocytogenes

→ Gram positive cocci bacillus

→ Tumbling motility

→ Blood agar, chocolate agar, Palcam agar (Cold enrichment method) at 4 c

Tests

→ Anton Test

→ Catalase test

Anton Test

Listeria culture into eyes of rabbit



Rabbit develops Keratoconjunctivitis

In newborn causes granulomatosis infant septica

In children & adults → Meningitis.

Pregnant female (Refrigerated Food)

Chorioamnionitis

PROM

Preterm birth

Abortions

DOC → Ampicillin

Gram Negative Bacilli

Enterobacteriaceae → GNB, catalase +ve, oxidase -ve, ferment sugar & produce acid with gas.

All are catalase +ve except catalase -ve *Shigella dysenteriae* type 1.

E. Coli

→ UTI

→ Diarrhoea

→ Meningitis

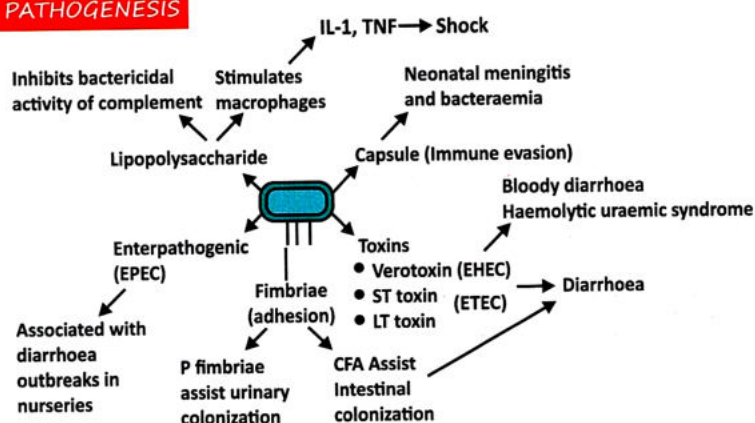
→ Scombroid fish Poisoning

UTI → > 10^5 E. coli/ ml of urine called significant bacteriuria

If on culture plate there are > 2 types of colonies

↓
Contamination

Sample → Midstream Urine. Sample Taken

E.coli PATHOGENESIS**Leukocyte esterase Test****Screening test for UTI****Catalase Test**

Culture is not a screening test.

Diarrhea

EPEC → Enteropathogenic *E. coli* causes pediatric age group diarrhea A/E Lesions (Attaching to villi damaging them effacing)

EIEC → Resembles shigellosis

→ Sereny Test: +ve

ETEC → Traveler's diarrhea

EHEC → Secrete Verotoxins (Slinga like toxins)

→ O157:H7 serogroup → Hemolytic Uremic Syndrome (HUS) in children

Culture media – SMAC (Sorbitol Macconkey Agar)

S. EAEC → Persistent type of diarrhea

→ Traveler's diarrhea (Few cases)

→ Stacked brick appearance on Hep 2 cell lines

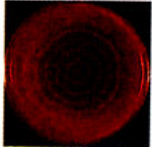
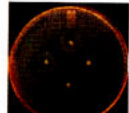
E. Coli having (K1 antigen)
[Neonatal meningitis]

E. coli

	<i>E.Coli</i>	<i>Klebsiella</i>
I - indole → → Indole	+	⊖
M - methyl red →	+	⊖
Vi- Voges proskauer test →	⊖	+
C- citrate →	⊖	+

Klebsiella

- Capsulated, non-motile urease ve
- *Klebsiella pneumoniae* (Cause pneumonia with features → Fried Lander's Bacillus)
- Red currant jelly sputum
- Bulging fissure sign
- *K. Ozaenae* → Atrophic Rhinitis (Roomy nose / Anosmia)
- *K. Rhinoscleromatis* → Rhinoscleroma (Woody nose)
- Mikulicz cells → Russel bodies seen
- Klebsiella granulomatis*
- Donovanosis (Granuloma inguinale)
- Dono van bodies seen
- Klebsiella* strain – Metallo Beta Lactamase -1
- T/t → Carbapenems
- On macconkey agar – Lactose fermenter (Pinkish mucoid colonies)

<i>Proteus</i>			
Swarms 	Form struvite stones (staghorn calculus) → Fishy smell → OXK ++++ : Scrub typhus (weil felix test)	Diene's phenomenon 	OXK, 19, 2 ↓ Cross react with rickettsiae family members

Salmonella Typhi

- Typhoid fever
- Faeco – oral contamination
- Step ladder pattern fever
- Rose spots
- Longitudinal ulcers

LD → B → Blood culture

A → Agglutination (Widal)

S → Stool culture

U → Urine culture

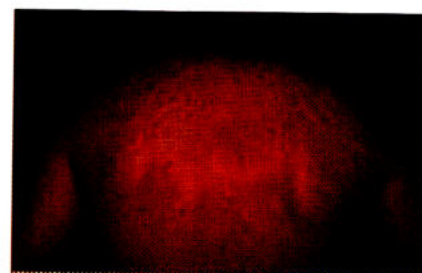
Blood Culture 1:10

(5 ml : 50 ml)

↓ ↓

Blood Bile broth (Culture Fluid)

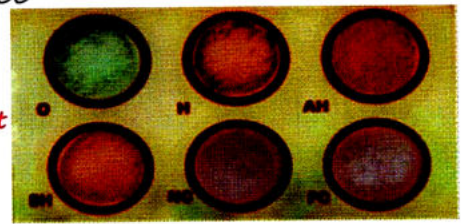
- Add SPS (Sodium polyanethol sulfonate) to blood culture bottle, it removes the antibiotic effect of blood acts as a anticoagulant



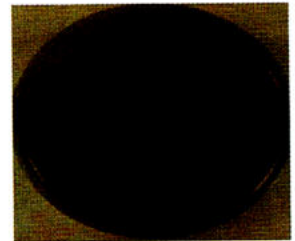
Rose Spots

- Widal Test (Agglutination test) → Highly sensitive
- Poorly specific
- Here are measured antibody titres against 'O' 1:100 & 'H' → 1:200
- > 1:100 'O' } Significant
- > 1:200 'H' }
- Typhidot (IgM testing)

Widal Test



- Stool & urine culture → Enrichment media (Liquid media) → Selenite F broth.
- Best selective media → Wilson & Blair (black colonies)



Non Salmonella Typhi

So Typhimurium, associate with poultry bird product contamination. (Campylobacter Jejuni also)



Gastroenteritis

S. Choleraesuis – septicemia

DOC → Ceftriaxone

Shigella

- Non motile; S. Dysenteriae



- Exotoxin / Endotoxin (Shiga toxin)
- Shigella dysenteriae type I → Catalase ⊖ ve
- MCC of bacillary dysenteriae in India → S. Flexneri
- MCC of bacillary dysenteriae in world in S. Sonnei (Late lactose fermenter)
- Transport media same as → Sach's buffered glycerol Saline
- Culture media → Same as Salmonella

Non Fermenters

- Pseudomonas
- B. Mallei
- B. Pseudomallei
- Acinetobacter

Pseudomonas

- GNB
- Catalase / Oxidase +ve
- Endo / Exotoxin A,S
- P. Aeruginosa produces pyocyanin (Bluish green) & pyoverdine (Green)
- Cx media – Cefrimide agar (Selective)

Diseases

1. Shanghai fever
2. Malignant otitis externa
3. Ecthyma Gangrenosum
4. VAP – Ventilator associated pneumonia
5. Burn wound infection

Antipseudomonal Drugs

- Vancomycin is not anti pseudomonas
- Polymyxin B & colistin – no resistance reported.

B. Mallei

- Glander's disease (Horse)
- Non motile

B. Pseudomallei

- Leads to Melioidosis
- Called Whitmore bacilli / Vietnam bomb
- Motile

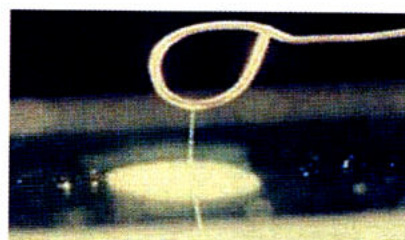
B. Cepacia → Infection cystic fibrosis patients

Acinetobacter Baumannii

- MDR isolated of ICU
- Oxidase – ve
- It is GNCB (Gram Negative Coccobacilli)

Vibrio Cholerae

C → Catalase	+ve
O → Oxidase	
I → Indole	
N → Nitrate reduction	
S → Sucrose lysis string Test	



It Has O 1 Antigen

	Classical	Eltor
Mukherjee phase IV sensitivity	⊕	⊖
Poly myxin 'B' sensitivity	⊕	⊖
Chick erythrocyte agglutination ve		

Classical	Eltor
→ Ogawa	→ Ogawa
→ Inaba	→ Inaba
→ Hikojima	→ Hikojima

Non O1 Vibrio → O2 → O139

(NAG) – Non agglutinable vibrios

Cholera Toxin

A + B → B helps in binding to GM1 ganglioside receptor



ADP ribosylation of G stimulatory protein



↑ cAMP



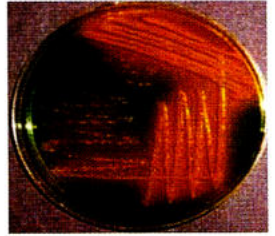
Intracellular water into lumen



Rice watery stool ^o(Mimic arsenic metal poisoning)

Culture Media

- Transport media → Venkateshwar Raman media (VR)
 → Cary Blair media
 Enrichment media → Alkaline peptone water
 Best selective → TCBS → (Thiosulfate citrate bile salt sucrose Green → Yellow)
 Darting motility



Halophilic Vibrios

- V. Parahaemolyticus (7%)
 → V. Vulnificus (8%)
 → V. Alginolyticus (10%) → Most Halophilic
 → V. Parahaemolyticus → Kanagawa phenomenon on wagatsuma agar
 → Sea food poisoning
 → V. Vulnificus → Coral cuts infection.

Haemophilus

H. Ducreyi → Cause Chancroid – painful genital ulcer

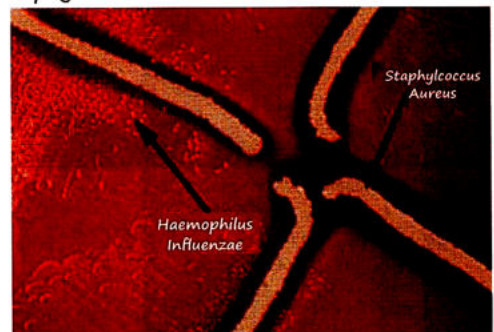


School of fish appearance or Tram track appearance on Tissue biopsy

H. Influenzae

Capsulated	Non Capsulated
↓	↓
Invasive infections	→ Sinusitis
→ Meningitis	→ Otitis media
→ Epiglottitis	

- Vaccine Prepared From Type 'B' PRP (Polyribitol phosphate)
 → Satellitism Seen



H. Aegyptius

- Pink eyes
 → Brazilian purpuric fever

H → H. Arophilus, paraprophilus, para influenza

A → Aggregati bacter

C → Cardiobacterium hominis

E → Eikenella corrodens

K → Kingella Kingae



Cause culture negative endocarditis

B. Pertussis

- Whooping cough (100 day cough)
 → Culture media → Bordet gengou media → Bisected pearl appearance

Or

- Aluminum paint app or Thumbprint appearance
 → Cough plate method

ICP → 1 – 2 weeks

→ Catarrhal → Most infections phase

- Whoops → Paroxysmal phase
- Acellular Pertussis vaccine used nowadays
- DOC → Macrolides

Brucella

- Brucellosis (Zoonotic disease)
- *B. Melitensis* – Sheep, goat, camel
- ↓
- Transmission happen by contaminated meat & milk
- By aerosols
- Acute Brucellosis (Undulant fever) [Malta fever, Mediterranean fever]

LD – Blood culture

- In Blood culture add erythritol – It Stimulate growth of Brucella
- Tbilisi phage typing done

SAT → Standard agglutination test

DOC → Doxycycline + Rifampicin

- For contaminated milk test
- Milk ring test
- Rose Bengal curd test
- Whey agglutination test

Plague

- *Yersinia Pestis*
- Cultured in ghee broth (Stalactite type colonies)

Types → Bubonic → 2 – 7 days

→ Septicemic → 2 – 7 days

→ Pneumonic → 1 – 3 days

→ Bubonic Plague → Flea bite transmits

Blood smear + Bubo smear

Wayson stains

Bipolar stained appearance

DOC → Streptomycin

Legionella Pneumophila

- Aerosols (Centralised AC)
- Water ingestion (More by water ingestion)

L. Pneumophila

Pontiac fever

Legionnaire's pneumonia

↓

↓

Self limiting

Which complicates to Legionnaire's disease

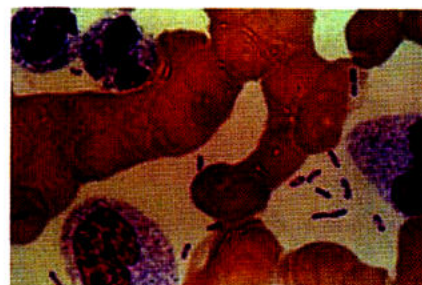
Culture media → Buffered Charcoat Yeast extract (BCYE)

→ In Alcoholics, smokers; infections of legionella is common

DOC → Macrolides

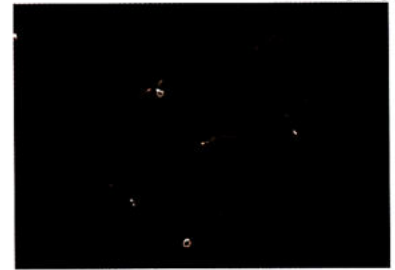
Spirochetes

- Dark ground microscopy used to demonstrate (Reflected light)



BCYE

- Levaditi & Fontana staining
- Syphilis → *T. Pallidum*
- ICP → 9 – 90 days
- Primary syphilis → Hard, painless, chancre
- Secondary syphilis → Rash over palms / soles
- Latent syphilis → 5 – 10 years
- Tertiary syphilis → Gummas
 - Cardiovascular syphilis → Aneurysm of aorta
 - Neuro syphilis (Tabes dorsalis)



Non Treponemal Test	
VDRL	RPR
Slide Flocculation test	
Need microscope	Card test
Not economical	Economical
CSF → Yes	CSF → No

Treponemal Test

- FTA → ABS → Fluorescent treponemal Ab assay
- TPI → *T. Pallidum* immobilization assay
- TPPA → *T. Pallidum* particulate agglutination
- Most sensitive test → FTA → ABS
- Most specific → TPI
- VDRL CSF > FTA ABS CSF
- Congenital syphilis → IgMFTA – ABS
- DOC → Benzathine Penicillin
- For Neurosyphilis → Penicillin G
- For Neurosyphilis → Penicillin G
- T. Pertunae* → Yaws
- T. Carateum* → Pinta
- T. Endemicum* → Bejel (Endemic syphils)

} Non STD

Borrelia

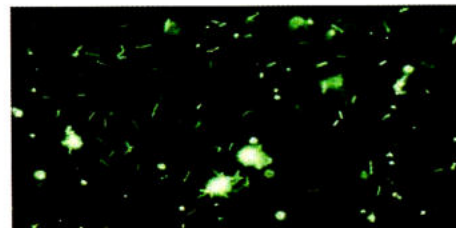
- Epidemic louse borne relapsing fever
- *B. Recurrentis*
- Epidemic tick borne relapsing fever
- *B. Duttoni*
- B. Burgdorferi* (Vector – Tick)
- Lyme's disease erythema chromium migrans (Bull's eye lesions)
- Culture media: Kelley's media
- B. Vincenti* → Vincent angina (Trench mouth)

Leptospira

- *L. interrogans* → Jaundice with Hemorrhagic fever
- Disease called Weil's disease

Lab Diagnosis

- Cx media → EMJH → Fletcher → Korthokoff
- MAT → Microscopic agglutination test.



Rickettsiae

- Epidemic typhus → *R. Prowazekii* → (Arthropods, Louse)
- Endemic typhus → *R. Typhi* → (Flea)
- RMSF → *R. Rickettsi* → (Tick)
- Indian Tick typhus → *R. Conorii* → (Tick)
- R. Pox → *R. Akari* → (Mite)
- Scrub typhus → *Orientia tsutsugamushi* → (Mite)

Scrub Typhus

- Black eschar
- Hilly regions
- C/F → Fever myalgia
- OXK ++++

DOC → Doxycycline

Q Fever

- *Coxiella burnetii*
- No rash
- No arthropod transmission
- Frequently contaminates milk (Killed by flash method pasteurisation)

Bartonella

- B. Bacilliformis* → Oraya fever
- B. Henselae* → Cat scratch disease

↓

Bacillary angiomatosis (HIV +ve)

B. Quintana → Trench fever

Ehrlichia Chaffeensis → Human Monocytic Ehrlichiosis

Ehrlichia Phagocytophilum → Human granulocytic ehrlichiosis

Chlamydia

2 forms known as ATP parasite

- Elementary body (Extracellular Form) → Infectious
- Reticulate body (Intracellular Form)

↓

Replicating

C. Trachomatis

- A, B, Ba, C → Trachoma
- D – K → Infantile pneumonia
- Genital chlamydiasis
- Inclusion conjunctivitis
- L1, L2, L3 – LGV (Lymphogranuloma Venereum)
- C. Psittaci* → Psittacosis
- Bird pneumonia

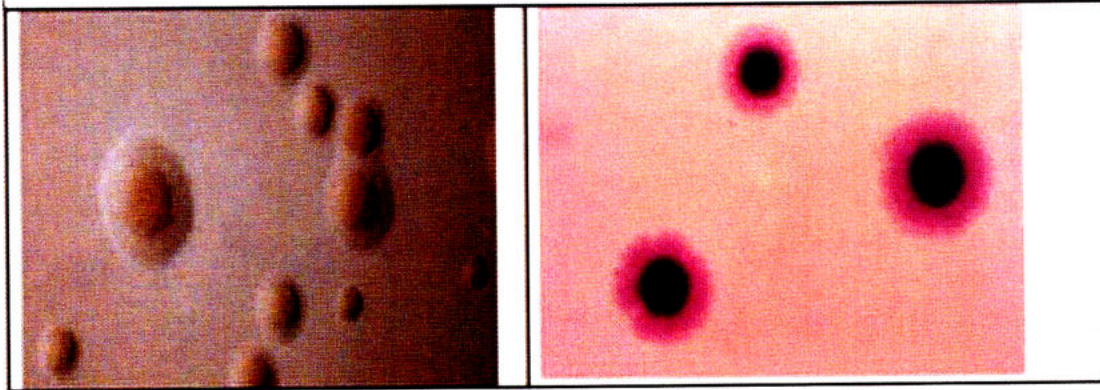
- C. Pneumoniae* → Adult pneumonia
→ Associates with Atherosclerosis

IOC → NAAT

Culture on McCoy cell line.

Mycoplasma

- No cell wall
- Cell membrane → Have sterol
- Grows on PPLO agar – has fried egg appearance



Fried Egg Colonies

M. Pneumoniae → MCC → Atypical Pneumonia

Dx → CAT → Cold agglutination test

M. Hominis & *genitalium*

↓

Non gonococcal urethritis (NGU)

MC cause → *C. Trachomatis*

Miscellaneous

- *C. Jejuni* → Poultry bird product contamination

↓

Inflammatory diarrhea

- In Children → Diarrhoea episode are F/B Guillain Barre syndrome
- Cultured on skirrow media – Charcoal based media
- Grow at 42 C

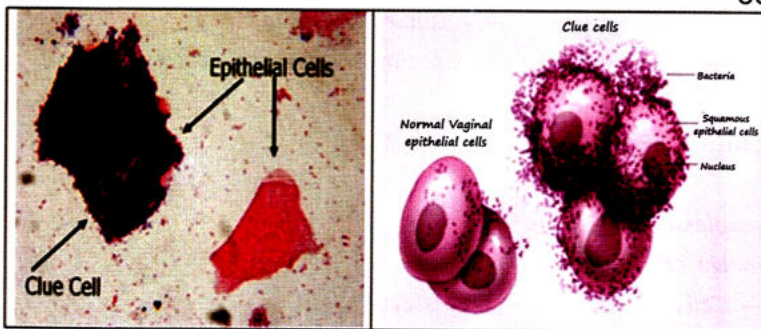
H. Pylori

- Leads to → PUD (Peptic Ulcer Disease)
 - Maltoma
 - Ca stomach
 - Non invasive test → Urea breath test.
 - Invasive test → Endoscopic biopsy from antrum & stain with warthin starry silver stain.
- ↓
- Demonstrate *H. pylori*
- Urease test → Urease media colour changes
 - From Yellow → Pink

Bacterial Vaginosis → *Gardnerella Vaginalis*

Amsel's Criteria

- pH > 4.5
 - Clue cells
 - Discharge + 10% KOH → Fishy smell
 - Nugent's Criteria
-
- Dog / Cat bites → *Pasteurella Multocida*
 - Human bites → *Eikenella corrodens*
 - *Bacteroides* → Brain abscess
 - GNB, Anaerobe
 - Mild potent endotoxin, may lead to shock & DIC
 - Scrombroid fish poisoning
 - *E. coli* > *Morganella* > *Proteus*
 - There is histamine production which leads to Vasodilation & Bronchoconstriction



Mycology

Morphological Classification

- Yeast → *Cryptococcus neoformans*
- Yeast like → *Candida* (Yeast, yeast like hyphae, pseudohyphae)
- Molds → *Aspergillus*, *rhizopus*

Dimorphic Fungus

- Mold → 25 degree C
- Yeast → 37 degree C

Dimorphic Fungal Disease – HSBCEP2

- mnemonic
- H** → Histoplasmosis
 - S** → Sporotrichosis
 - B** → Blastomycosis
 - C** → Coccidioidomycosis
 - P** → Para coccidioidomycosis
 - P** → Penicilliosis

Fungus	In vitro (25° C)	In Vivo (37° C)
Blastomyces	 Mold	 Yeast
coccidioides	 Mold	 Spherule
Histoplasma	 Mold	 Yeast
Paracoccidioides	 Mold	 Yeast
Sporothrix	 Mold	 Yeast

Sexual Spores: ZAB

- Z - Zygo spores
- A - Ascospores
- B - Basidio spores

Asexual

- A - Anthroconidia
- B - Blastomyces
- C - Chalmydospores

Based On Location

Superficial Mycosis

- *Tinea versicolor*
- *Tinea migha*
- Dermatophytes

Subcutaneous Mycosis

- M → Mycetoma
- R → Rhinosporidiosis
- C → Chromoblastomycosis
- S → Sporotrichosis

Systemic Mycosis / Deep Mycosis

- HSBP2
- Candida
- Cryptococcus

Culture Media → SDA (Sabouraud's dextrose agar)
→ pH = 5.4 – 5.6

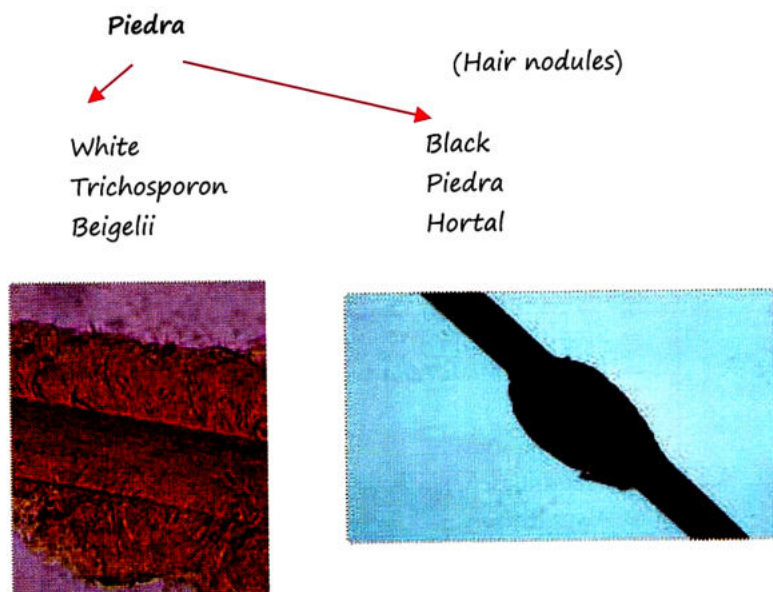
Fungal Stains

- Indian ink stain
- Mucicarmine stain
- Nigrosin
- GMS (Gomori Methamine Silver)
- LPCB (Lactophenol Cotton Blue)
- H&E (Haematoxyllin + Eosin)
- Calcofluor white – Fluorescent stain

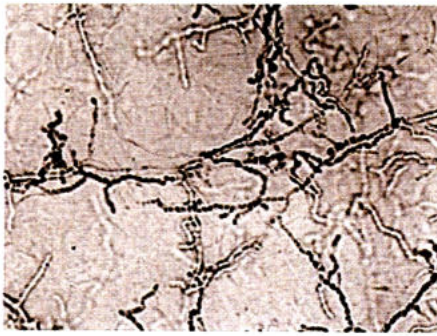
T- Versicolor

- Caused by *M. Furfur* / *M. Globosa*
- Sphagetti & meat ball appearance
- Fried egg colonies (lipophilic fungus) on SDA
- Golden yellow fluorescence

T. Nigra → *Exophiala werneckii*



Dermatophytes



	Skin	Hair	Nails
Trichophyton	✓	✓	✓
Microsporum	✓	✓	X
Epidermophyton	✓	X	✓

LD → Culture growth on SDA + LPCB

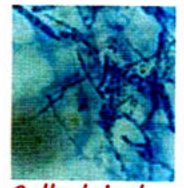
	Macroconidia	Microconidia
Trichophyton	Pencil shaped	Numerous
Microsporum	Spindle shaped	Less
Epidermophyton	Club/boat shaped	Absent microconidia



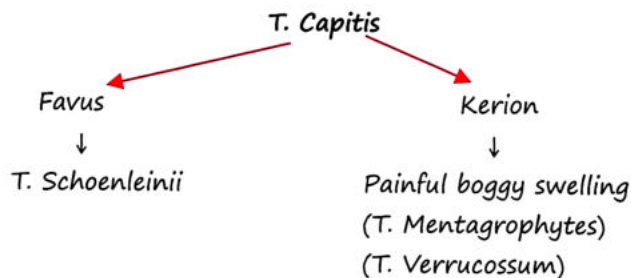
Boat shaped
macro conidia



Spindle
macroconidia



Cylindrical
macroconidia



Favus by
T. schoenleinii
T. violaceum
T. sypseum



T. Cruris → Dhobi itch or jock itch

T. Pedis → Athlete's foot

Mycetoma

- Sinus
- Swelling
- Granules (Discharging)



Actinomycetoma	Eumycetoma
→ Numerous sinus & swelling	→ Single sinus & swelling
→ Color of granules – Yellow /white except in Actinomadura pelletri where they have red granules	→ Black → Madurella mycetomatis
	→ White
	→ Acremonium
	→ Pseudallescheria boydii
→ Responds to antibiotic	→ Amputation

Rhinosporidiosis

Caused by *rhinosporidium seeberi* (Aquatic protist-protozoa)

- B/L nasal polyp
- Polyp biopsy → Spherule
- Within spherule there are endospores
- Non cultured on SDA
- T/t → Surgical treatment



Chromoblastomycosis

- Colorful fungus
- Dermatiaceous fungus

Eg

- Alternaria
- Bioplaris
- Curvularia
- Phialophorea
- Cladosporium
- Exophiala werneckii
- Thorn prick / wood worker

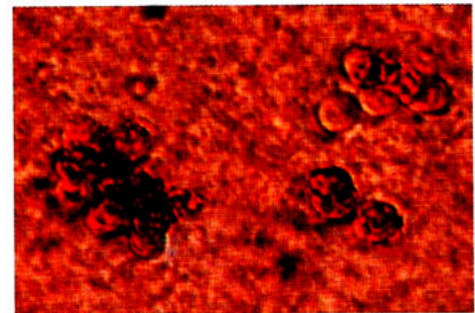


Verrucous lesion



On tissue biopsy + H& E staining

- Medlar bodies
- (Brown colored sclerotic bodies)
- (Copper penny / muriform appearance)



Sporotrichosis

- Sporothrix schenckii
- Rose gardner's disease
- Lymphocutaneous spread

- Dimorphic → Mold → Flower like sporulation
- Yeast → Cigar shaped yeast cell (Narrow based budding)
- Asteroid yeast cells



Sporothrix schenckii

Histoplasmosis

- Darling's Disease
- Histoplasma capsulatum
- Aerosols → Pulmonary involvement



Blood



Visceral involvement

- D/D of TB

Mold → Tuberculate macroconidia

Yeast → Narrow based budding

Blastomycosis

→ North American's disease / Gil Christ

→ Yeast cell → Figure of '8' appearance or broad based budding yeast cell

→ Can happen in HIV + person

Coccidioidomycosis

Caused by *Coccidioides immitis*

San

Joaquin

Valley

Fever

Desert

Rheumatism

Mold → Arthro conidial arrangement

Yeast → Spherule with endospore

Paracoccidiomycosis

(South American disease / LN_2 mycosis)

↓

Yeast cell → Mickey mouse appearance / Mariner's wheel appearance

Penicilliosis

→ *Penicillium marneffei* (HIV +ve)

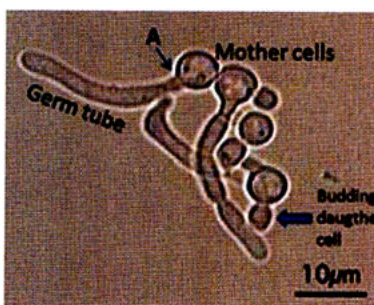
→ Brick reddish color pigment

→ Brush conidial arrangement

Candida

Reynold Brande
Phenomenon (Germ tube

Chlamydospore formation on corn meal
agar (Nutrient deficient media)



C. Krusei → Inherent resistant to azoles

C. Auris → Worldwide infections in immunodeficient individuals



oral thrush

Candida Albicans

Diseases

- Oral thrush
- Oesophageal candidiasis
- Vaginal candidiasis (Curdy white discharge)
- Onychomycosis (Nail folds)
- Candidaemia
- Candiduria
- Meningitis

Predisposing Factors

- Steroids use, immune deficient individuals, neutropenic patients > HIV +ve
- CMC (Chronic mucocutaneous candidiasis) (T cell disorders)

LD

- Cultured on SDA → Creamy & pasty colonies
- Chrome agar → Colorful fungus
- PCR for *C. Albicans*
- For Invasive candidiasis → β D glucan assay

β D Glucan Assay

C → Candidiasis

A → Aspergillosis

P → *Pneumocystis carinii*

Cryptococcus

- True yeast
- In immune deficient (HIV +ve)
- Pulmonary infections

↓

Blood

↓

Meninges (Meningitis)

→ Co Meningitis

LD → Blood & CSF → Cultured on SDA

→ Smooth mucoid creamy colonies

→ Phenyl oxidase enzyme in *Cryptococcus* – responsible for melanin production

→ On niger seed agar – Brown black colonies are seen

Latex Card Agglutination Test for Cryptococcal Antigen

PCR for *C. Neoformans*

DOC Cryptococcal meningitis → LAMB + Flucytosine

→ Capsulated organism → Indian ink staining
(Polysaccharide)

↓

Virulence factor



Zygomycetes → Seen in Terminally ill patients

R → Rhizopus

A → Absidia

M → Mucor

→ Rhinocerebral zygomycosis

→ Tissue biopsy → H & E stain



→ Aseptate hyphae

→ Obtuse angled

→ Biopsy material on SDA



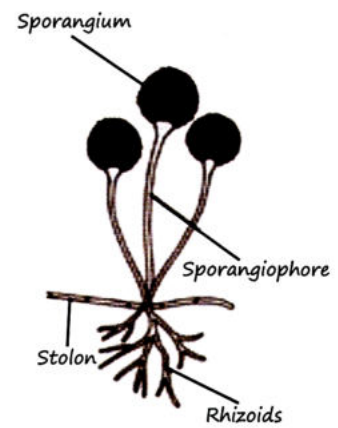
Culture growth + LPCB



Rhizopus

Absidia - Inter nodal rhizoids arrangement

Mucor - No rhizoids



Mucor Mycosis Is Commonly Seen In

→ DM

→ Frequent blood transfusion

→ Chronic dialysis

Aspergillosis → Common in DM, immunodeficient

A. Fumigatus

→ Leads to allergic broncho pulmonary aspergillosis (ABPA)

→ Associates with aspergilloma (Fungal balls)

→ Oculomycosis

→ Otomycosis

→ On SDA → Greenish color colonies in SDA

A. Niger

→ Blackish crust from ear

On SDA → Black colored colonies

A. Flavus → Produces Aflatoxin which leads to hepato cellular carcinoma

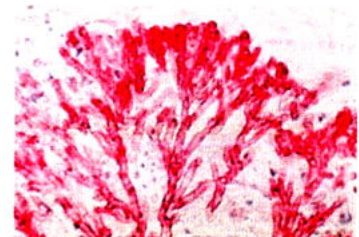
On Tissue Biopsy + H & E Staining

→ Septate hyphae

→ Dichotomous branching

→ Mostly Angle → 45°

Dichotomous branching of Aspergillus



Ergototoxicosis

→ Claviceps purpurea seeds along with edibles.

→ St. Anthony's fire or St. Vitus dance

Occupational Diseases

Disease	Organism
Farmer's disease lung	→ Hay dust → <i>Thermoactinomyces vulgaris</i> → <i>Micro monospora faenii</i>
Baggassosis	→ Sugarcane dust → <i>Actinomyces sacchari</i>
Maltster's lung	→ Barley & malt factories → <i>A. Clavatus</i>
Cheeser's lung	<i>Penicillium Caseocolum</i>

Pneumocystis Carinii / *P. Jirovecii*

- Protozoa → That exists in cyst & trophozoite form
- Fungus → Chromosomal studies favour

PC Pneumonia

- Occurs when CD 4 < 200 in HIV +ve
- DOC cotrimoxazole

Lab Dx

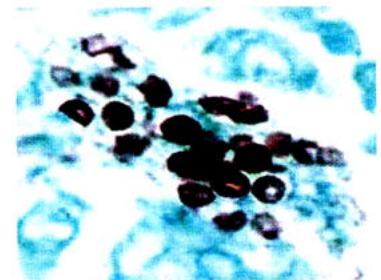
- BAL Fluid
- Gastric washings

↓

Stained with GMS

↓

Cyst – Black (Because of glycogen)
Trophozoite – Green



Parasitology

Protozoa

Entamoeba Histolytica

- Infective Form → Quadrinucleate cyst (ingested with contaminated food and water).
- Cyst Excystation → Trophozoites (actively motile with eccentric nucleus)

Intestinal amoebiasis
(Pain, diarrhea, dysentery)

Extra intestinal amoebiasis
MC Site → Liver > lungs > Brain

↓

In The Liver

MC site → Posterior superior portion of Right lobe of liver damage to hepatocytes leads to Anchovy sauce pus/ chocolate pus

IOC → EIA / ELISA

Intestinal Amoebiasis → Trophozoites go to liver but sometimes come to stool

Stool Examination Is Done

Saline Mount → Made In normal saline for trophozoites which are motile by false pseudopodia.

Erythrophagocytosis (engulfed RBCs) is seen

(Characteristic feature)

Charcoal leyden crystals are seen

Iodine Mount → Made for cyst

- have 4 nuclei (Quadrinucleate)
- 1 or 2 nuclei cyst also come in stool with Chromatoidal bars & Glycogen Vacuoles

→ Cultured on media → PCR media (Philips craige's Robinson)

→ Balamuth's media

→ PCR (polymerase chain reaction) for *Entamoeba Histolytica*

→ Flag shaped ulcers seen in dysentery caused by *Entamoeba Histolytica*

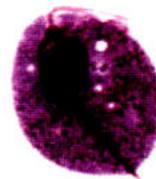
Free Living Amoeba

Naegleria Fowleri	Acanthamoeba	Balamuthia
→ Causes PAM (Primary Amoebic meningoencephalitis) → Infective form – amoeboid form → Resides in fresh water pond → H/O swimming / fresh water pond → Flagellated forms – enter nares – cribriform plate – brain – PAM → So called “Brain eating Amoeba”	→ Causes encephalitis → Leads to Contact lens keratitis ↓ Optic route ↓ Granulomatous encephalitis → Aerosol route – lungs – brain – granulomatous encephalitis	→ Causes granulomatous Encephalitis

Vaginal Flagellate

Trichomonas Vaginalis

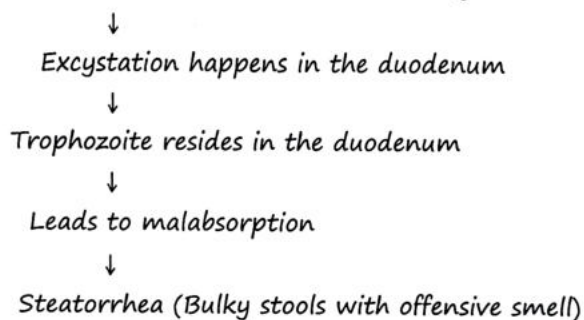
- Doesn't have a Cystic stage
- Only trophozoite stage seen
- Disease caused is Trichomoniasis:
 - pH > 4.5
 - Greenish frothy discharge
 - Colpitis Macularis (Strawberry vagina and cervix)
- DOC → Metronidazole given to both partners as it is a STD



Pap Smear Showing *Trichomonas*

Intestinal Flagellate Giardia Lamblia

Infective form – Quadrinucleate / Binucleate cyst



The Trophozoites

- Tennis Racquet shaped
- Have 4 pairs of flagella
- Axostyle
- Sucking disc
- Falling leaf like motility
- Entero test / string test to detect trophozoites
- CVID → Common variable immunodeficiency (in which B cells are not able to mature into plasma cells) A/W giardiasis
- DOC → Metrogyl



Haemo-Flagellates

1. *Leishmania* → Infective form → Promastigote
 2. *Trypanosoma* → Infective form → Metacyclic Trypomastigote
- Leishmania Donovanii* → Kala Azar (Visceral leishmaniasis)
Leishmania Tropica → Oriental sore / Delhi Boil / Baghdad boil / Aleppo button
Leishmania Braziliensis → Espundia / Mucocutaneous Leishmaniasis

Kala Azar

- Vector → Sandfly
- C/F → Hyperpigmentation + fever
- Hepato-splenomegaly +nt



Lab Dx

- Splenic biopsy (More sensitive and specific) > bone marrow biopsy:
- Amastigote forms / aflagellar forms seen
- A/k/a LD bodies (Leishman Donovan bodies)
- Blood examination → Anemia + Leukopenia + Thrombocytopenia
 - Hyper gammaglobulinemia: detected by Napier's Aldehyde test / chopra's antimony test.
- rk-39 antigen testing (card test)
- Culture on NNN (Novy macNeil Nicolle - Promastigote Grown)
- DOC → Liposomal AMB



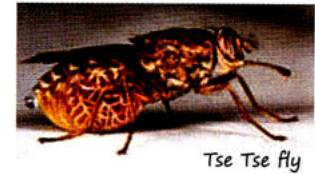
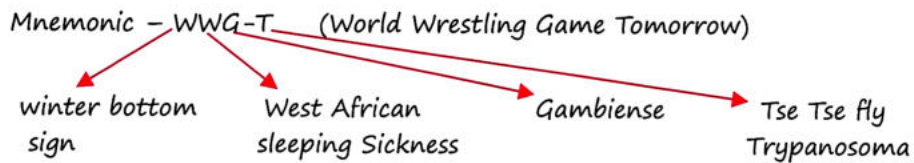
PKDL

- After 2 years of successful treatment
- From Viscerotropic, it becomes Dermotropic
- Nodules all over the skin
- Tt → Sodium stibogluconate



Trypanosoma Brucei → Causes sleeping sickness (African trypanosomiasis)

Gambiense (Tse Tse Fly/Glossina)	Rhodesiense
"West African Sleeping Sickness"	"East African Sleeping Sickness"
→ Winter Bottom Sign – (Cervical Lymphadenopathy)	
→ Kerandel sign	

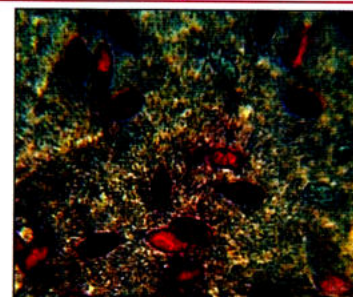
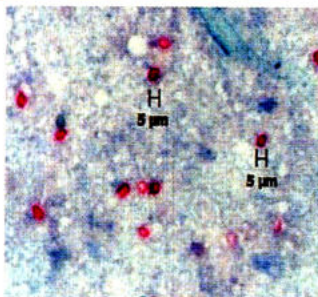


T. Cruzi

- Chagas disease (American Trypanosomiasis)
- Transmitted by → Reduviid bug
- Swelling k/a chagoma (Entry site oedema)
- Romana sign (Eyelid swelling)
- Megaesophagus
- Mega colon
- Myocarditis
- Meningoencephalitis

Coccidian Parasites

	<i>Cryptosporidium Parvum</i>	<i>Cyclospora</i>	<i>Isospora Belli</i>
A/W	HIV	HIV	HIV
Oocyst size	4 – 6 μ	8 – 10 μ	23 – 36 μ
DOC	Acid fat in nature Nitazoxanide	Acid fat in nature Cotrimoxazole	Acid fat in nature Cotrimoxazole

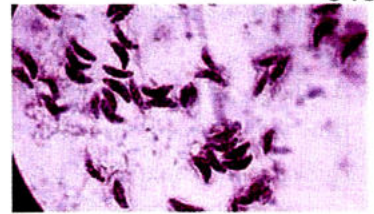


Note → Auto infection happens in cryptosporidium parvum.

Note → Microsporidium bieneusi → In HIV +ve → Diarrhoea

Toxoplasma Gondii

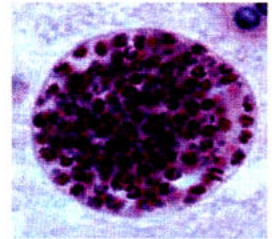
- Definitive Host → Cat
- Intermediate Host → Rat/Man
- Enteric Cycle – in Cat, there is formation of oocyst
- Exoenteric Cycle – in Rat/Man, there is no oocyst formation



Tachyzoites

Modes Of Transmission

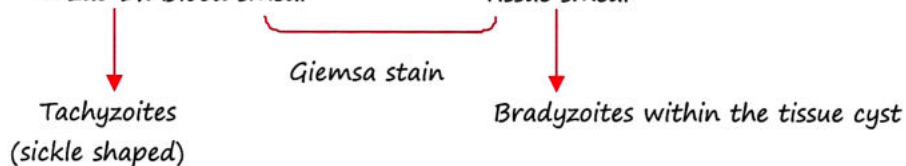
- Contaminated food & water with Sporulated oocyst
- Transfusion of blood infected with Tachyzoites
- Eating contaminated meat having tissue cyst containing Bradyzoites
- Oocyst is not infective for man. Only Sporulated oocyst is infective.
- Toxo in normal individual – Mild cervical Lymphadenopathy
- But in HIV +ve patients – encephalitis
- Toxoplasmosis in pregnancy
 - 1st Trimester → Max damage to the foetus
 - 3rd trimester → Max chance of transmission



Bradyzoites

Congenital Toxoplasmosis

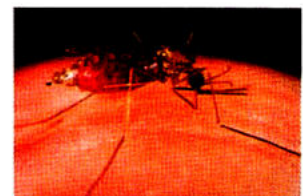
- Chorioretinitis, calcifications in brain and convulsions
- Microcephaly / Macrocephaly along with mental retardation
- Lab Dx Blood smear Tissue smear



- Sabin Feldman dye Test (Complement fixation test)
- PCR / ELISA for TORCH panel
 - Toxoplasma
 - Rubella
 - CMV
 - Herpes
 - Syphilis
 - Varicella zoster

Plasmodium

- Infective form in Man – Sporozoites
- in Transfusion induced Malaria, infective form – Trophozoites > merozoites
- Definitive Host – FAM (Female anopheles mosquito)
- Intermediate Host – Man
- World malaria day → 25th April
- Conditions which prevent P. Falciparum infection
 - G → G6PD deficiency
 - S → Sickle cell anemia
 - T → Thalassemia trait
 - Excess of fetal Hb
- Condition preventing against P. vivax



Female Anopheles

→ Duffy negative RBC.

Lab Dx

1. Blood Smear

Thin blood smear for - Speciation

Thick blood smear for - prevalence of parasite

P. Vivax	P. Falciparum
→ Young age RBC are involved	→ All age RBC are involved.
→ ↑ in size of RBC	→ Normal size RBC.
→ Schuffner's dot	→ Maurer's dot
→ Gametocyte stage	→ Banana shaped Gametocyte.
→ Schizont stage	→ Present but Absent from blood Smear.
→ Single Ring within RBC	→ Multiple Rings within RBC.
→ Accole forms Absent	→ Accole forms present
→ Relapse (hypnozoites)	→ Recrudescence d/t incomplete Rx
→ Reddish Brown Pigment	→ Dark brown pigment

Mnemonic A → All aged RBC

M → Maurer's dot

B → Banana shaped gametocyte

A → Accole forms

R → Renal failure / Recrudescence

P. Malariae

Z → Ziemann's dot

O → old age RBC

O →

M → Max ICP

I →

N → Nephrotic Syndrome

→ BAND Forms are present in P. Malariae.

2. Fluorescent Microscopy (Buffy coat examination) using acridine orange

3. Malaria Card Test → HRP-2 (histidine rich protein) is present in the cards of P. Falciparum

4. LDH and Aldolase based kits for other Plasmodium species

5. PCR for plasmodium species

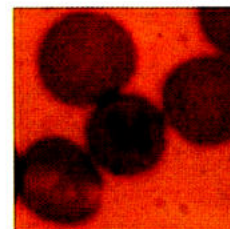


Malaria Card Test

	P vivax	P falciparum	P malariae	P ovale
Ring forms (early trophozoites)				
Developing trophozoites				
Immature schizonts				
Mature schizonts				
Micro-gametocytes ♂				
Micro-gametocytes ♀				

Babesia Microtii

- Tick borne
- Intra erythrocytic protozoa
- Maltese cross → Mimics *P. Falciparum* gametocyte stage (Banana shaped)

**Maltese Cross****Balantidium Coli (Ciliate)**

- Largest Intestinal Protozoa
- 2 types of nucleus - Micro/Macronucleus
- Cytostome /cytopharynx
- Pig to Pig Transmission or Pig to man transmission
- Diarrhoeal episodes
- DOC → Tetracycline (Doxycycline)

**Helminthology**

Cestodes	Trematodes	Nematodes
→ Tape like → Monoecious (Both male and female in one) → Suckers with hooks. → Alimentary canal Absent → Body cavity absent	→ Leaf like → Monoecious except schistosomes (Dioecious) → Suckers without hooks → Absent to Poorly developed → Absent	→ Cylindrical → Dioecious → Buccal Capsule → Well developed → Present

Cestodes

	Definitive Host (DH)	Intermediate Host (IH)
T. Solium	Man man	Pig man
T. Saginata	Man	Cattle
E. Granulosus	Dog	Sheep/Man
H. Nana	Man	Man
D. Latum	Man	1 st IH → Cyclops 2 nd IH → Fish.

Diphyllobothrium Latum (Fish Tapeworm)

- Infective form – L3 Plerocercoid Larvae +nt in fish which is eaten by man and gets the infection
- Operculated egg (Lid)
- a/w Megaloblastic anemia

**H. Nana (Dwarf Tapeworm)**

- Infective form → Egg
- Egg has polar filaments, polar thickening with central hooklets
- Non bile stained egg
- Autoinfection → Present
- DOC → Praziquantel



Non Bile Stained EggN → *Necator Americanus*E → *E. Vermicularis*H → *H. Nana*A → *Ancylostoma duodenale***H. Diminuta (Rat Tape Worm)**

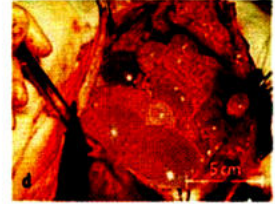
→ (Autoinfection → absent)

→ (Egg lacks polar filaments)

Echinococcus Granulosus

→ Infective form – eggs with contaminated food and water

→ MC site – liver (Hydatid cyst) > lungs > brain

**Hydatid Cyst** → Most active layer – endocyst (Germinal epithelium)

→ It leads to formation of brood capsules, protoscolices and hydatid fluid. This can be detected by Casoni's test (Type 1 HS reaction)

→ Best treatment is surgical removal.

→ PAIR therapy

Echinococcus Multilocularis (Malignant Hydatid Disease)**Tinea**

T. Solium	T. Saginata
	(Longer)
Armed (Rostellum with Hooklets)	Unarmed tapeworm as it lacks Rostellum with Hooklets
	
Larval form A/K/a Cysticercus cellulosae	Larval form A/K/a Cysticercus bovis
Eating Uncooked pork leads to taeniasis diarrhoea	Eating uncooked beef leads to taeniasis diarrhoea
Infective form → Eggs majority of cases of Cysticercosis Also for Neuro-cysticercosis: → Mc → Subarachnoid → Presents with Atypical Seizures → IOC → CT /MRI → DOC → Albendazole	No infection with eggs of taenia saginata

Trematodes (Leaf Like Flukes)

- Monoecious
- 2 intermediate host
 - 1st → Snail
 - 2nd → Aquatic vegetation / Fish / Crab
- Infective form → Metacercariae (Cyst like structure)
- Operculated egg (lid) except Schistosomes → Infective form – Cercariae
 - Single IH → 1st – snail
 - Dioecious
 - Non operculated egg

Schistosomes

- Dioecious
- IH → Snail
- Infective form → Cercariae
- Non operculated egg

S. Haematobium (Resides in vesical plexus)

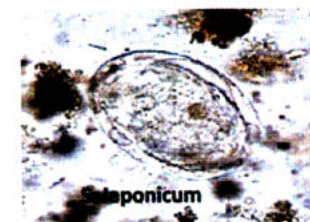
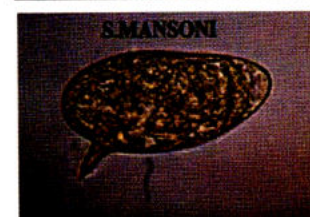
- SCC of urinary bladder
- Terminal hematuria
- Egg has a Terminal spine

S. Mansoni

- Resides in the inferior Mesenteric Plexus.
- Katayama fever
- Swimmer's itch (Cercarial dermatitis)
- Egg has a Lateral spine

S. Japonicum

- Resides in Superior Mesenteric plexus
- Katayama fever
- Eosinophilic Diarrhoea
- Egg has a Lateral knob / rudimentary spine



Fasciola Hepatica (Sheep Liver Fluke)

Infective form – metacercariae

1st IH – Snail

2nd IH – aquatic vegetation. (Metacercariae) → (Man eats aquatic vegetation & acquires this)

In bile duct larvae matures to adult forms → Eggs are passed in faeces.

Clonorchis Sinensis (Oriental / Chinese Liver Fluke)

1st IH → Snail

2nd IH → Fresh water fish. (Metacercariae)

↓

Larvae matures in bile ducts to adult forms

↓

Constant irritation & inflammation of Bile duct

↓

Carcinoma bile duct

Paragonimus Westermani (Lung Fluke)

1st IH → Snail

2nd IH → Crab / cray water fish (Metacercaria)

→ Rt lung → Erosions l/t Red brown sputum. (Endemic hemoptysis)

Fasciolopsis Buski (Intestinal Fluke) → Largest intestinal trematode

Nematodes

Small Intestinal	Large Intestinal	Tissue Nematodes (Lymphatics)	Subcutaneous Sites
Ascaris	Trichuris trichiura	W. Bancrofti	Loa Loa
Ancylostoma Duodenale	E. Vermicularis.	Brugia malayi	Onchocerca volvulus
Necator			
americanus			
S. stercoralis			

Infective Form

By Eating Eggs

E → Enterobius vermicularis

A → Ascaris

T → Trichuris trichiura

By Penetration Of Filariform Larvae In Sole Of Foot

A → Ancylostoma duodenale

N → Necator americanus

S → Strongyloides stercoralis

Lungs For Maturation

A → Ascaris

A → Ancylostoma Duodenale

N → Necator Americanus

S → S. Stercoralis

Large Intestinal Worms

T. Trichiura (Whip Worm)

→ Barrel shaped (dumbbell shaped) egg with mucus plugs.

→ Bile stained

→ Rectal prolapse in children (coconut cake like rectum)

→ Growth retardation

→ A/W Fe deficiency anemia



Enterobius Vermicularis (Pin/ Seat/ Thread Worm Or Oxyuriasis)

→ Perianal pruritus in children (MC) → Autoinfection:

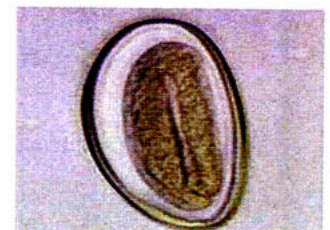
→ Egg → D shaped

→ Has tadpole like larvae

→ Plano convex egg

→ Non bile stained

→ NIH swab used



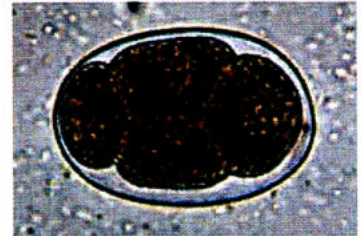
Autoinfection

- C → *Capillaria Phillipinensis*, *Cryptosporidium Parvum*
- H → *H. Nana*
- E → *E. Vermicularis*
- S → *S. Stercoralis*
- T → *Taenia Solium*

Small Intestinal Worms

Ancylostoma Duodenale (Hook Worm)

- Infective form – Filariform larvae (enter sole of foot)
 - Maculopapular dermatitis (ground itch)
 - Lungs for maturation
 - *A. Caninum* (Seen in kennel workers)
 - *B. Brasiliensis*
 - *Uncinaria stenocephala*
 - *phlebotomum*
 - Wakana Disease (GI disease)
 - A/W Fe deficiency anemia
 - Egg is segmented with blastomeres
 - Non bile stained egg
- } Cutaneous larvae migrans



Strongyloides Stercoralis

- Infective form – Filariform larvae
- Ovo- Viviparous
- A/W HIV +ve
- Parthenogenetic development → Female lays eggs without male.
- Autoinfection (Hyperinfection syndrome in HIV+ve pts CNS and viscera are involved)
- Cutaneous larvae migrans (Larvae currens)

Ascaris

- Infective form → **Fertile Egg** → A/W Intussusception; can l/t Intestinal obstruction
- Lungs for maturation (2nd & 3rd moulting of larvae)
- Löffler's syndrome (Massive eosinophilia)
- Female → Egg (Unfertilized egg)

→ Eggs → Float on saturated salt solution.

- F → Fertilised egg of ascaris
- A → *A. Duodenale*
- T → *T. Trichiura*
- E → *E. Vermicularis*
- H → *H. Nana* infertile Egg



Fertile Egg



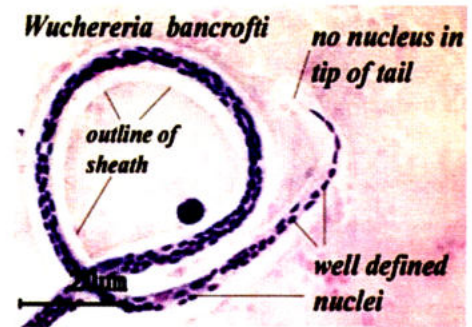
infertile Egg

Tissue Nematodes

W. Bancrofti

- Infective form – L₃ filariform larvae
- Vector → Culex
- Viviparous (Microfilariae) → Nocturnal periodicity (Come in blood at night)
 - Sheathed (No nuclei in tail tip)

Acute Filariasis → Fever, Lymphadenitis, Lymphangitis



Chronic Filariasis → LN sclerosed and blocked so leads to

- Elephantiasis
- Hydrocele
- Chyluria in urine
- Albuminuria
- Granuloma Breast in females

Lab Dx → Day time testing – DEC given which irritates the

larvae and come in blood at daytime and Blood smear examination

done → Sheathed microfilariae of W. bancrofti with no nuclei in tail tip seen whereas Brugia Malayi microfilariae is sheathed but has terminal 2 nuclei in tail tip.



→ IOC → PCR for W. Bancrofti

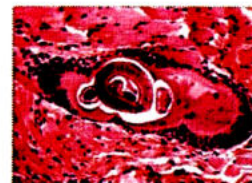
(Even Picograms of DNA can be detected)

Subcutaneous Site Filariae

	Skin	Eye	Fly
Onchocerca volvulus	Onchocercoma (S/C nodules) Leopard skin	River blindness	Simulium fly or black fly
Loa- Loa	Calabar swellings	Loasis	Chrysops fly

Trichinella Spiralis / Muscle Worm

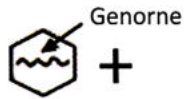
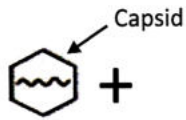
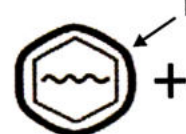
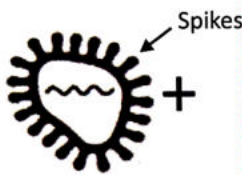
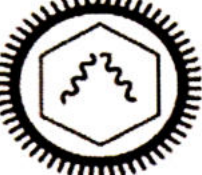
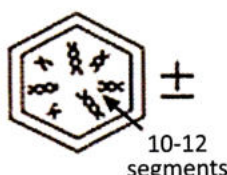
- Infective form → Larvae in uncooked pork
- Viviparous



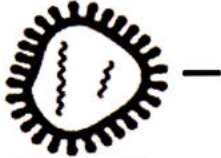
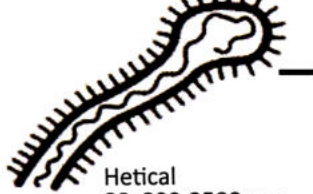
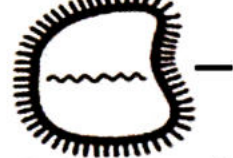
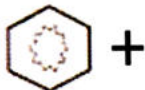
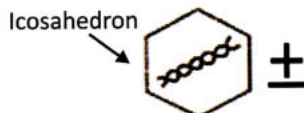
Dracunculus Medinensis (Guinea Worm Disease)

Visceral Larvae Migrants

- Toxocara canis
- Toxocara cati
- Angiostrongylus cantonensis (also a/w eosinophilic meningitis)

Cutaneous Larvae Migrants→ *A. Caninum*→ *A. Brasiliensis***RNA viruses****Picornaviurs**C = 32
22-30 nm**Astrovirus**C = 32?
30-35 nm**Calicivirus**C = 32 (holes)
35-39 nm**Flavirus**Icosahedral
45-50 nm**Togavirus**Icosahedral
70 nm**Coronavirus**Pieomorphic
120-160 nm**Retrovirus**Icosahedral
90-120 nm**Reovirus**C = 132
60-80 nm**Bunyavirus**

90-120 nm

OrthomyxovirusHelical, Pieomorphic
80-120 nm**Arenavirus**Pieomorphic
110-130 nm**Filovirus**Helical
80x800-2500 nm**Rhabdovirus**Helical
60x180 nm**Paramyxovirus**Helical, Pieomorphic
150-300 nm**DNA viruses****Circovirus**Icosahedral
17-22 nm**Parvovirus**C = 12
18-26 nm**Hepadnavirus**C = 160 Icosahedral
40-48 nm**Papovavirus**C = 72
45/55 nm**Adenovirus**C = 252
75-80 nm**Herpesvirus**C = 162
150-200 nm**Poxvirus**Complex
240x300 nm

DNA Viruses	Shapes
H ² → Herpes	→ Bullet → Rabies
Hepadna	→ Space vehicle → Adeno
A → Adenovirus	→ Filamentous → Marburg & Ebola
P → Parvo	→ Brick → Pox
P → Pox	
P → Papova	
Y	

→ Viruses are either DNA or RNA but never both.

→ Nucleic acid surrounded by capsid / Capsomeres

Symmetry

→ Icosahedral (MC)

→ Helical

→ Complex (pox)

→ DNA viruses are ds, except parvo which is ss DNA

→ RNA viruses are ss, except ds RNA Reovirus.

Replication

→ DNA virus → nucleus except pox

→ RNA virus → Cytoplasm except Influenza & HIV

Non Enveloped Rna Viruses

P → Picornaviridae: Hep A, Enterovirus 72

A → Astroviridae

R → Reoviridae

C → Caliciviridae: Hep E

Non enveloped are more resistant

Non Enveloped DNA Viruses

P → Parvo

A → Adeno

P → Papova

Non Enveloped Rna Viruses

P → Picornaviridae: Hep A, Enterovirus 72

A → Astroviridae

R → Reoviridae

C → Caliciviridae: Hep E

Note → Hep A & E are non-enveloped RNA

Culture Media

→ Hen's egg

→ Cell lines

→ Animal inoculation

→ Hen's egg inoculation is called candling

→ Viruses growing in chorionic allantoic membrane (CAM) ~ Variola / Vaccinia

→ Allantoic cavity

Amniotic Sac

Influenza virus

→ Yolk Sac C → Chlamydia
 A → Arbo
 R → Rickettsiae

→ New born suckling mice (age < 48 hours)
 → Arbovirus
 → Coxsackie virus

Cell Lines

→ (Primary cell line) PCL – 5-10 divisions
 → (Diploid cell line) DCL – 10- 50 divisions
 → (Continuous cell line) CCL – Unlimited divisions

PCL → Eg. Rhesus monkey kidney cell lines
 → Chick embryo fibroblast cell line

DCL → Eg. Human fibroblast cell line

CCL → Eg. HeLa, Hep2, BHK, McCoy cell line.

Inclusion Bodies (Replicating aggregates)

Intracytoplasmic

Negri bodies	→ Rabies virus
Paschen bodies	→ Variola
Guarneri bodies	→ Vaccinia
Molluscum	→ Molluscum contagiosum
(HP bodies	
Henderson	
Patterson)	

Intranuclear

Cowdry Type A

H → Herpes
 A
 Y → Yellow fever

Cowdry Type B

B
 A → Adeno
 P → Polio

Both Intranuclear & Intra cytoplasmic – Measles (Warthin Finkeldey Giant Cells) and Cytomegalovirus.

Viral Interference (If one is growing other one not allowed to grow)

Rubella → Togaviridae

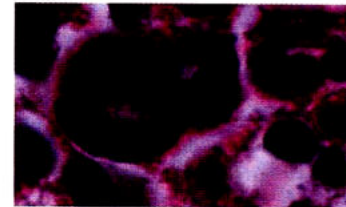
Polio → Picornaviridae

Herpes Zoster/ Shingles

Ophthalmicus	Oticus
Single sided lesion	Ramsay Hunt syndrome
Dermatomal involvement T ₅ - L ₃	Bell's palsy along with vesicles
	Over external
	Auditory canal
Hutchinson rule is seen. (if tip of nose involved then cornea will be involved)	
Ophthalmic branch of Trigeminal nerve	Geniculate ganglion

CMV (HHV5)

- Largest herpes virus
- Owl eye appearance of infected cell
- Congenital CMV
- MC feature → Hepatosplenomegaly
- Best sample is urine.



CMV Is Associated With

- Mononucleosis like syndrome → Atypical lymphocytes (Paul Bunnell test - ve)
- Post kidney transplant infections (CMV Pneumonia)
- CMV pneumonia
- CMV retinitis at CD4 count < 50
- DOC - Ganciclovir
- PCR for CMV
- Culture in Human fibro blast cell line

EBV (HHV - 4)

- Has affinity to B cells (CD21/CR2)
- Infectious mononucleosis
- Nasopharyngeal carcinoma
- A/W Burkitt's lymphoma (Starry sky appearance)
- A/W HL/ NHL
- Duncan Syndrome (X linked lymphoproliferative syndrome)
- Tonsillar Carcinoma
- Oral hairy leukoplakia (HIV +ve)
- Infectious Mononucleosis (Kissing Disease)
 - Fever
 - Hepatosplenomegaly
 - Atypical lymphocytes (CD 8)
 - PAUL BUNNELL Test +ve
 - PCR for EBNA & VCA

HHV 8

- Kaposi sarcoma (Vascular tumor) in HIV +ve
- Castle man's disease
- Primary effusion lymphoma

Von Magnus Phenomenon

High haemagglutination titre but low infectivity

Herpes α - Virinae	β - Virinae	γ - Virinae
HSV - 1	HHV 5 (CMV)	HHV - 4 (EBV)
HSV - 2	HHV 6	HHV - 8 (Kaposi sarcoma causing virus)
HHV - 3 (Varicella zoster)	HHV 7	

HSV

- HSV - 1 Above waist
 - HSV - 2 Below waist
- } Not always true

HSV- 1

- Temporal lobe encephalitis
- Cutaneous Infections → MC Site- Face
- Eczema Herpeticum
- Herpetic whitlow → Doctors & Nurses
- Herpetic gladiatorum → Wrestlers
- Optha → Acute dendritic ulcer
- C/I → Steroids
- Indicated → Topical acyclovir

HSV - 2 Genital

- Pregnant female gets HHV 2
- ↓ Caesarean section
- Reduce Transmission to newborn
- PCR/ ELISA
- Tzanck Smear (for HSV 1, 2, 3)

HHV 3 (Chicken Pox)

- IP → 14 - 16 days (2-3 weeks)
- SAR > 90%
- Infectivity period - 2 days before rash to 5 days after the rash or until the scab falls
- Dew drops on rose petal is seen like lesion.
- MC complication → Secondary bacterial pneumonias
- Congenital Varicella Syndrome (Rare)
 - Chorioretinitis
 - Microcephaly
 - Cicatrising lesions
 - Limb hypoplasia

If a Pregnant female acquires the infection around the delivery time ie 5 days before to 2 days -The newborn has to be given immunoglobulins to prevent infection

HHV 6, 7 →

Exanthem subitum or Roseola infantum

Pox Virus**Molluscum Contagiosum Virus (MCV)**

- Pearly white umbilicated nodules over skin (Children) / genital (as STD in adults)
- MCV - 1 (MC)
- MCV - 2 (In HIV +ve, MCV 2 is more common)
- On Tissue biopsy – Molluscum bodies (Intra cytoplasmic eosinophilic inclusion bodies) – Henderson Patterson bodies (HP Bodies)

T/t → Cryosurgery along with podophyllin resin

Adenovirus

- Space vehicle shaped
- Type 3, 4, 7, 14
- Leads to pharyngo conjunctival fever
- Type 3,7,14 leads to swimming pool conjunctivitis.
- AAV (Adeno associated virus) + Vaccinia (Adeno associated virus) are used as a vector to produce recombinant vaccines
- Hep B Vaccine (Recombinant killed vaccine) prepared in yeast.

Parvo B 19

Associates with

- Aplastic anemia
- Non Immune Hydrops fetalis
- Poly arthralgia syndrome
- Erythema Infectiosum (Slapped cheek appearance ~ 5th disease)

**Papovaviridae**

- HPV 1,2,4 → Common warts
- HPV 6,11 → Genital warts, Laryngeal warts
- HPV 6, 11, 16, 18 → Ca cervix
 - Vaccine Gardasil → Quadrivalent 6, 11, 16, 18
 - Cervarix → Bivalent 16, 18
 - Gardasil 9 → HPV 6, 11, 16, 18, 31, 33, 45, 52, 58)
 - Act on Early Proteins → E6 & E7 (Target)

JC Virus → Progressive multifocal leuco encephalopathy (PMLE)

Bk Virus (Mnemonic: Borrowed Kidney)

- Infection common in Post kidney transplant
- BK Nephropathy – Decoy cells

SV - 40

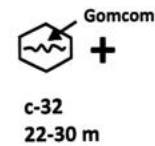
- Simian Vacuolating virus 40
- Cytoplasmic Vacuolation

RNA Viruses

Picornavirus

- Polio virus
- Coxsackie virus
- Enterovirus
- ECHO (Enteric cytopathogenic human orphan)
- leads to aseptic meningitis
- Rhinovirus – common cold virus

Picornavirus



polio virus
coxsackie
enterovirus
echovirus
rhinovirus
hepatitis A

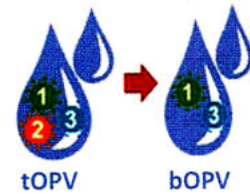
Polio Virus

Type I → MC

Type II → Most antigenic

Type III → VAP (Vaccine associated paralysis)

- Polio vaccine → (Trivalent → Bivalent) Type II is removed called switch.
- Transmission route → Faeco-oral
- > 95% infections → In apparent
- < 1% infections → Paralysis occurs (Anterior horn cells are damaged)
- Histopathology Findings
 - Nissl body degeneration in cell
- Tonsillectomy is contraindicated in a polio epidemic
- Culture → Monkey kidney cell lines
- ELISA for polio virus
- PCR for polio virus



COX SACKIE – A

Leads To

1. Herpangina (Acute Vesicular Pharyngitis)
2. Acute haemorrhagic conjunctivitis (Enterovirus 70 + Coxsackie virus A 24)
3. HFMD → Hand foot mouth disease (Coxsackie virus A 16)
4. Aseptic meningitis

COX SACKIE – B

- Bornholm's disease (Epidemic pleurodynia) (A/K/a Devil's grip)
- DM; HFMO & aseptic meningitis
- Culture → On Newborn suckling mice (Age < 48 hours.)

Enterovirus

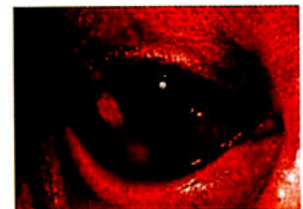
- EV 70 → Acute haemorrhagic conjunctivitis
- EV 71 → HFMD
- EV 72 → Hep A

Orthomyxovirus

- Influenza A → 8 pieces of RNA
- Influenza B → 8 pieces of RNA
- Influenza C → 7 Pieces of RNA



Hand, Foot and Mouth Disease



Orthomyxovirus



Helical, Pleomorphic
80-120nm

Influenza

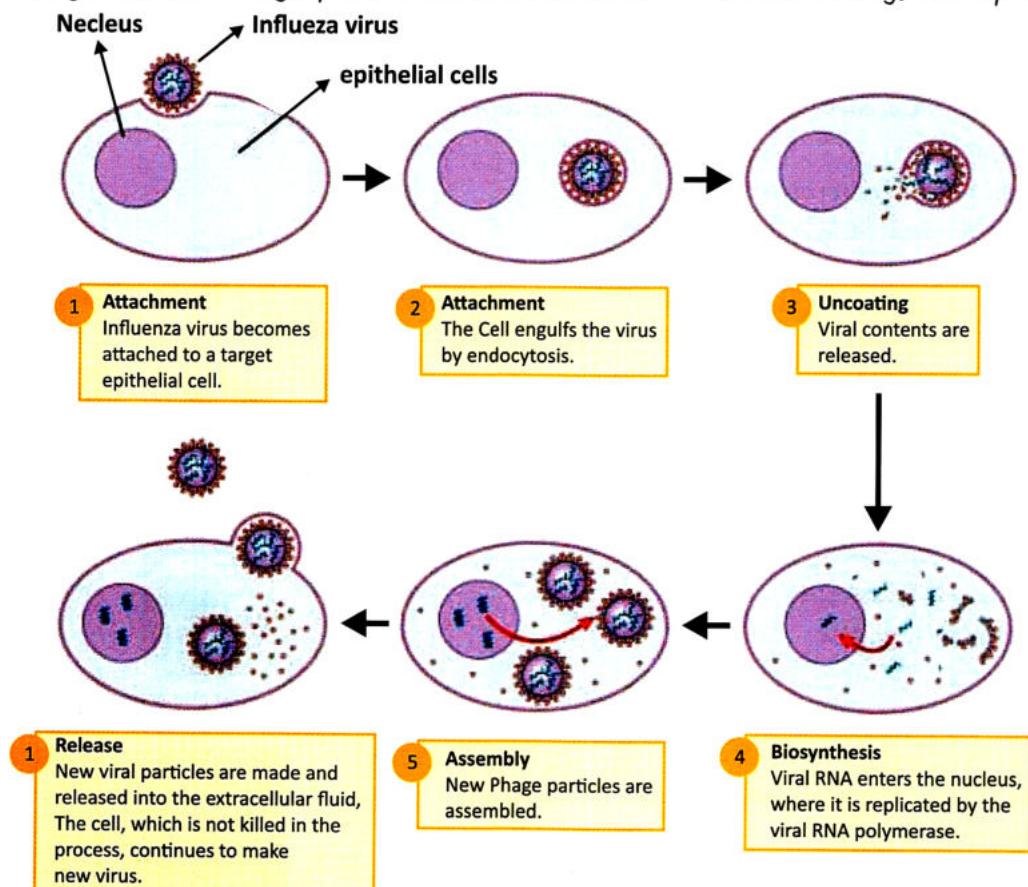
→ Segmented RNA seen in:

- B → Bunyaviridae
- I → Influenza
- R → Reoviridae
- A → Arenaviridae

Glycoproteins

- Haemagglutinin (H) → Adherence to the cell
- Neuraminidase (N) → RDE (Receptor Destroying enzyme)
- The process is called Elution

- H₁N₁ (Influenza A) suddenly changes to H₁N₁ called **Antigenic Shift**
- H₃N₂ (Inf. A) Changes to H₃N₂ called **Antigenic Drift**
- Antigenic Shift → Abrupt, drastic, discontinuous - pandemics majorly, associates with epidemics
- Antigenic drift → Single point mutation, continuous - Endemics initially, later epidemics



H₁N₁ → Human influenza strain

+
Avian Flu strain
+
Swine Flu strain

Lab Dx

- Nasopharyngeal swabs → Real time PCR [IOC]
- RNA → cDNA & then amplified.

Nasal Vaccines (Live) And Killed Vaccines Available

- DOC → Prophylaxis – Tamiflu (Oseltamivir)
- It is a negative sense RNA virus & it changes to positive sense RNA virus with the help of RNA polymerase

Paramyxovirus

- -ve sense RNA viruses
- 1. Parainfluenza 1, 2 → Croup (Acute LaryngoTracheobronchitis)
- 2. Mumps → B/L parotitis (MC presentation)
 - Sometimes meningoencephalitis can precede parotitis
 - SAR → 85%
 - ICP → 2 - 3 weeks
 - Young males → Orchitis
 - Young females → Oophoritis

Paramyxovirus



Parainfluenza
RSV
Mumps
Measles

Vaccine strain – Jeryl lynn strain

Measles / Rubella

- Characteristic feature – Koplik's spot (Opposite lower second molar)
- ICP → 10 - 14 days
- Infectivity period → 4 days before rash & 5 days after rash
- Isolation period → Prodromal features (10th day)

↓
Koplik spot (12th day)

↓
Rash (14th day)

i.e. Isolation period is from 10th day to 3rd day of Rash.

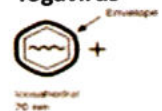
- Inclusion bodies: Intra nuclear + intracytoplasmic i.e. Warthin finkeldey giant cells.
- Chronic late complication SSPE (Sub Sclerosing Pan Encephalitis.)
- Edmonston zagreb vaccine strain

Lab Dx PCR/ ELISA for Rubella / Measles virus

Rubella

- Family Togaviridae
- Congenital Rubella syndrome
 - C → Cataract
 - H → Heart disease (PDA>PS)
 - D → Deafness (SNHL)
- Forchheimer spots sports over uvula
- Blueberry muffin rash + IUGR + thrombocytopenia called blueberry muffin syndrome.
- Infectivity Period → 7 days before the rash to 7 days after the rash.
- IP - 2 - 3 weeks
- Vaccine strain - RA 27/3

Togavirus



rubella
chickungunya

RSV

- Leads to acute bronchiolitis
- DOC → Ribavarin
- Fusion protein leads to formation of multi nucleated giant cells.

Nipah Virus

- Subfamily → Henipavirus
- Kerala → Coconut trees
- Reservoir → Fruit bats
- Amplifiers → Pigs (virus present in the secretions mostly in urine)
- Transmission → Eating & drinking contaminated food & water
- Contact with secretion of patient
- Mortality > 70%
- IOC → PCR for Nipah

Rabies Virus

- Sub family → Lyssa Virinae
- Family → Rhabdoviridae
- Travels at the speed of 3 mm/hr
- Two types

→ Natural virus → Furious rabies	Fixed virus → Dump rabies
Hydrophobia → +nt	-nt
Negri bodies → +nt	-nt

Antemortem Diagnosis

- Corneal smear
- Nape of neck – hair follicle

For both, DFAT (Direct fluorescent antigen testing) can be done

Postmortem Diagnosis → Negri bodies demonstrated in hippocampus by seller's technique.
(Intra cytoplasmic eosinophilic inclusion bodies)

Arbovirus

- Togaviridae → Chikungunya, WEE (Western equine encephalitis), EEE
- Flaviviridae → Japanese encephalitis (JE), Dengue, yellow fever, Hep C, Zika virus, KFD
- Reoviridae → Orbivirus (Colorado tick fever)
- Bunya viridae → Hanta Virus → Hantan pulmonary syndrome,
→ Hantan Renal syndrome
- Phlebo Virus → Associated with rodent fecal material
- Nairo Virus → Crimean-congo hemorrhagic fever
- Belgrade Virus
- Pumula Virus

JE

- Reservoir - Ardeid Birds, Egrets
- Amplifier - Pigs
- Transmitted by - Culex vishnui
- Culex Tritaeniorhynchus
- > 30% mortality



Dengue

- Vector – *Aedes aegypticus* / tiger mosquito
- Serotype 1, 2, 3, 4, 5 most dangerous – 2
- Most dangerous combination serotype 1 f/b 2

↓ Leads to

Dengue hemorrhagic fever

↓ If not treated

Dengue shock syndrome

This is Called Antibody dependent enhancement (Seen in Serotype 1 followed by 2)

- Break bone fever
- Lab Dx 1st 2 days: NS – 1 antigen detection by ELISA (Non specific/ by Non structural protein)

On 5th day – IgM detection by ELISA

- IOC – PCR for serotype. If serotype 1 detected then serious steps of prevention need to be taken to prevent type 2 otherwise serious complications like DHF/DSS can happen



Yellow Fever

- Vector – *Aedes*
- Yellow fever vaccine certificate – lifetime validity
- 17 D vaccine (Live)
- Only live vaccine which can be given to pregnant female
- 1st viral disease to be discovered.

Chikun Gunya

- Immense joint pain (Crippling joint pain)
- Never complicates like dengue

Kyasanur Forest Disease

- Reservoir – Rat/ Squirrels
- Amplifiers – Monkey
- Transmitted by – Hard Tick
- Mild haemorrhagic fever



Hepatitis

Hep A	Hep B	Hep C	Hep D	Hep E
picornaviridae	Hepadnaviridae	Flaviviridae	Dependent on Hep B	caliciviridae
(Route) Faeco-oral	Parenteral sexual vertical	Parenteral	-	Feco - oral
(ICP) 15 – 45	50 – 180 d	15 – 150 d	50 – 150 d	15 – 50 d
Chronicity				
-	1 – 10%	> 85%	-	-
Carrier	0.1 – 30%	1.5 – 3.2%	-	-
-				
Fulminancy	0.1% – 1%	0.1%5	-	20%1-2%
0.1%				↑ es to 20% in pregnancy

- Hep A → Only Hep virus to be cultured
- Hep B → Spherical (MC)
 - Tubular
 - Dane (Complete viral genome (42 nm in size))
- Hep B has 4 genes
 - P → Polymerase
 - C → Core
 - S → Surface
 - X → Carcinogenicity
- IgG anti HBC → comes early & persist late (Till last)
- HBs Ag + IgM antiHBC + HBc (Active replicating phase)
- HBs Ag → Australian antigen
- Anti HBs → Vaccinated
- > 6 months HBsAg → Chronic Hep B
- Hep B vaccine → Killed recombinant (Prepared in yeast)
- 0, 16 months
- Anti HBs titres > 10 IU/ml

Diagnosis

- Hepa card testing
- ELISA for Hep B
- PCR for HBV – DNA

Hepatitis C

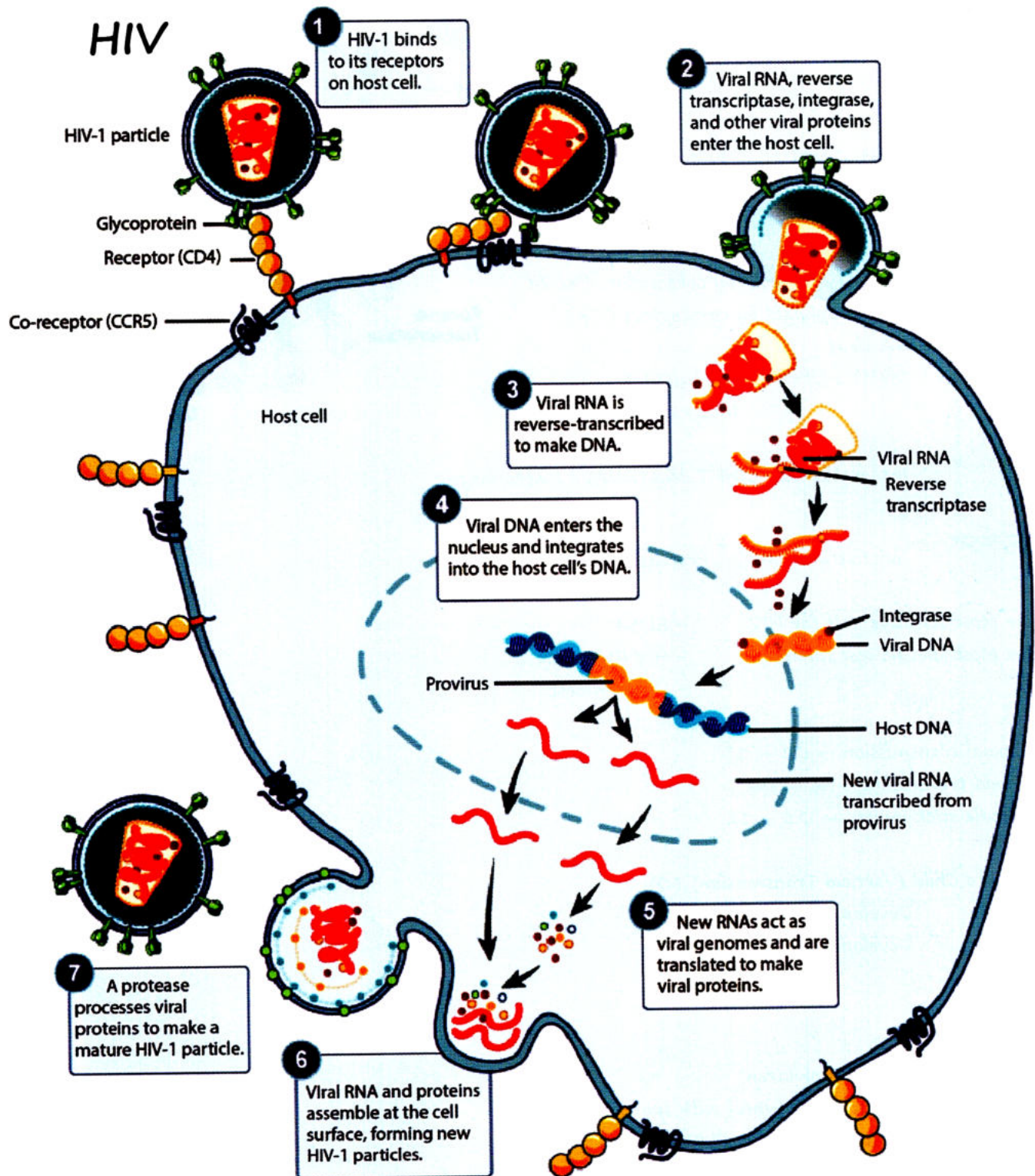
- Quasispecies (Antigenic variations)
- Therefore no vaccine
- Chronicity is maximum, so leads to cirrhosis (Rx liver transplantation).

Hepatitis D

- Hep D → Super infection (More dangerous)
 - Coinfection
- Hep B → Hep D superinfection has more fulminancy (5-20%)

Hep E

- 1 – 2% fulminancy
- But in pregnancy 20% fulminant



→ Family - Retro viridae

→ Subfamily → Lentivirinae

→ HIV - 1 >> HIV 2



Belong to group 'M' subtype 'C'



HIV - 1



3 structural genes → Gag - Matrix protein
 → Pol - Polymerase
 → Env - Envelop, Gp41, Gp120

→ HIV affects CD4+ve 'T' cells by coreceptors CXCR4

→ HIV affects macrophages by coreceptors CCR5

→ It is a ssRNA virus

→ ssRNA $\xrightarrow{\text{Reverse Transcriptase}}$ dsDNA
 $\xrightarrow{\text{Integrase}}$

Integrates with CD4 T cells (DNA) / Macrophage

Inactive proteins $\xrightarrow{\text{Protease}}$ Active proteins

→ Most sensitive Test for HIV

- ELISA

→ Most specific test for HIV

- HIV RNA PCR

- Western blot

Routes of Transmission

Sexual transmission → 0.1 - 1%

Blood transfusion → > 90%

Needle stick injury → 0.5 - 1%

Mother to Child (Vertical Transmission) 30%

Developing countries → 15%

Developed countries → 0-5%

Rota Virus

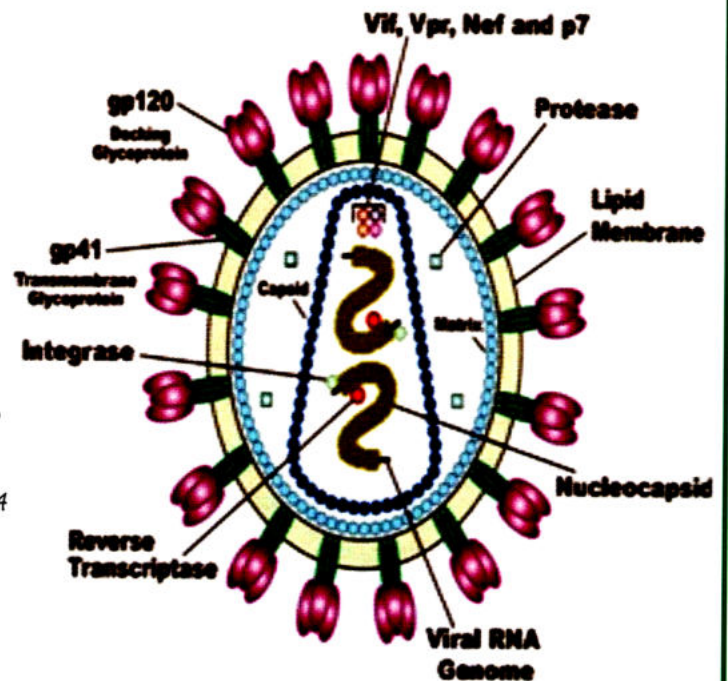
→ dsRNA

→ MCC of diarrhea is children

→ Electron Microscopy → Wheel with spoke appearance

→ IOC → Rota virus antigen by ELISA in stool sample

→ NSP - 4 (Non structural protein) → Secretory diarrhea



Zika Virus

Transmitted by → Sexual route

Mosquito → Aedes

- If Pregnant female gets infection → Baby can have Microcephaly
 - GBS (Guillain Barre Syndrome)
- C/F → Fever
 - Rash
- IOC → PCR for zika

EBOLA Virus

- Family → Filoviridae
- Filamentous shape
- Transmitted by contact with secretion of patient
- BSL-4 (Bio Safety level) maintained to handle
- IOC → Real time PCR (within 2 hours result are obtained).



Prions

- Prions are proteins
- PrP changes to → PrPsc due to abnormal folding
- Disease:
 - Kuru
 - CJD
 - Scrapie
 - Mad cow disease
- Prions are most resistant form on Earth.
- Denaturation occurs at 135 C X 1 hr (Auto clave)

Immunology

Innate Immunity Influenced By S → Species I → Individual R → Race	Acquired Immunity → B cells → T cells → APC (Antigen presenting cells) Classical complement pathway
	Example → Natural active → Infection → Natural passive → (Mother to child) → Artificial active → Vaccination → Artificial passive → Readymade Igs

Immune Response (IR)

Primary IR → Slow, Sluggish, Short lived → IgM mainly

Secondary IR → Prompt, Powerful, Prolonged, IgM → IgG (Isotype switching)
→ By change in heavy chain (Constant portion)

Antigen → Are foreign substances, If enter the body

↓

Leads to formation of antibody under proper conditions (pH, temperature)

Hapten → Incomplete antigen

→ When combine with carrier protein becomes immunogenic.

Factors Which Affect Immunogenicity of an Antigen

1. Nature of substance

Protein (Best Antigen) > carbohydrate > Lipids > Nucleic acids (Worst Antigen)

2. More molecular weight & valency, more immunogenic

3. Tissue enzyme response

4. Foreignness & immunogenicity

Superantigen (Mostly non MHC restricted)

Attaches to lateral portion of TCR (T - Cell receptor)

↓

Activate > 20% 'T' cells

↓

Massive lymphokines release

↓

Shock

Examples

→ Food Poisoning of *S. Aureus*

→ ET - A ~ Super antigen

→ SSSS (ETA, ET-B)

→ TSS (Pyrogenic toxin) [Enterotoxin F]

→ Scarlet fever toxin (A, C) - Pyrogenic / erythrogenic toxin of streptococcus

→ *Mycoplasma arthritidis*

→ *Malassezia Furfur*

→ *Yersinia Enterocolitica*

→ EBV, CMV, HIV, Rabies virus

→ *Enterococcus faecalis* - not a superantigen

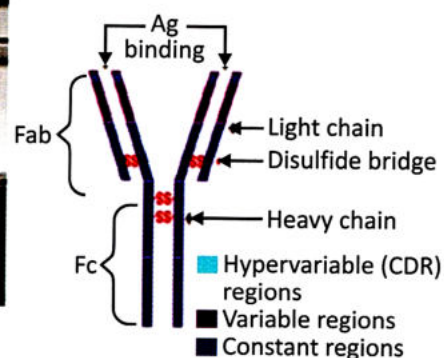
Antibodies

→ Antibodies are γ - Globulins



Gerald M. Edelman

Rodney R. Porter



$$1L = 1V + 1C$$

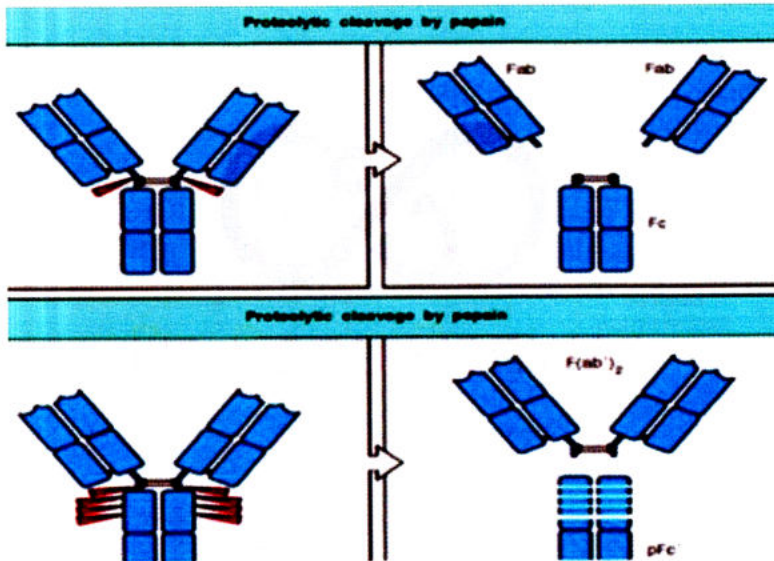
$$1H = 1V + 3C/4C$$

Ag binding sites are called hotspots

/ Hypervariable region / Paratope

Got nobel prize in medicine for
chemical composition of Ab. $2L + 2H$

Proteolytic Cleavage



→ On proteolytic cleavage by papain → Above hinge region → $2\text{ Fab} + 1\text{ Fc}$

→ On proteolytic cleavage by pepsin → Above hinge region → $1\text{ F(ab')}_2 + \text{Fc}'$

Immunoglobulins				
IgG	IgA	IgM	IgD	IgE

IgG

- Longest T $\frac{1}{2}$
- Max concentration
- Ig G₁, G₂, G₃, G₄
- MC - IgG₁
- Only Ig which passes through placenta, except IgG₂
- F portion of IgG plays a role in opsonization. (Enhancement of Phagocytosis)

IgA

- Monomer
- Dimer (J chains)
- IgA₂ → Serum
- IgA₁ → Body secretions
- MC selective Ig deficiency ~ IgA
- IgA with IgG deficiency leads to frequent capsulated organism infections.

IgM

- Maximum molecular weight
- Maximum sedimentation coefficient
- Pentamer (Valency = 10)
- Also plays role in opsonization.

IgE

- Shortest $t_{1/2}$
- Minimum concentration
- Role in homocytotropism (Movement towards mast cells) (Type 1 HS)
- Heat labile

Light Chains

- Kappa → Lambda 2:1
- Multiple Myeloma → Ig G light chains are involved

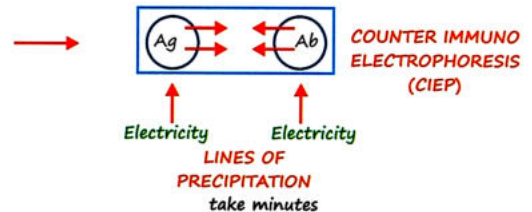
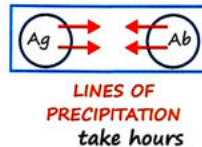
Antigen Ab Interaction → Happen in Zone of Equivalence – Perfect lattice formation

Precipitation → Soluble antigen + Ab → Insoluble precipitate

Eg → Ascoli's thermo precipitation test (For anthrax antigen)

Flocculation (Modification)

- Slide Flocculation Test – VDRL



- Single diffusion in one dimension (Oudin)
- Double diffusion in one dimension (Oakley Fulthorpe)
- Single diffusion in two dimensions (Radial Diffusion)
- Double diffusion in two dimensions (Ouchterlony)
- Rocket Electrophoresis → Precipitation reaction

Agglutination

- Insoluble antigens + ab → Clumps (Easily visualized)
- Agglutination >>> precipitation

Ex

- ABO blood grouping examination
- Widal test
- Mycoplasma Pneumoniae → CAT – Cold agglutination test
- Brucellosis → SAT – Standard agglutination test
- Leptospirosis → MAT – Microscopic agglutination test
- Direct coomb's test (In vitro test) for Fetal antibodies
- Indirect Coomb's test (In vitro test) for maternal antibodies
- Passive latex agglutination test – By addition of latex particle soluble antigen becomes insoluble Ag. (i.e. Precipitation → Agglutination)

Ex

- ASO
- CRP
- Rheumatoid factor test

Complement Fixation test

- Wasserman Reaction → Syphilis
- Sabin feldman dye test → Toxoplasmosis

Neutralisation Test (Toxin + Anti toxin)

- Nagler's reaction → α Lecithinase (Cl. Perfringens)
- Schick test → C. diphtheria

Immuno chromatographic test – on nitro cellulose paper

Eg → Hepa card

→ Pregnancy card test

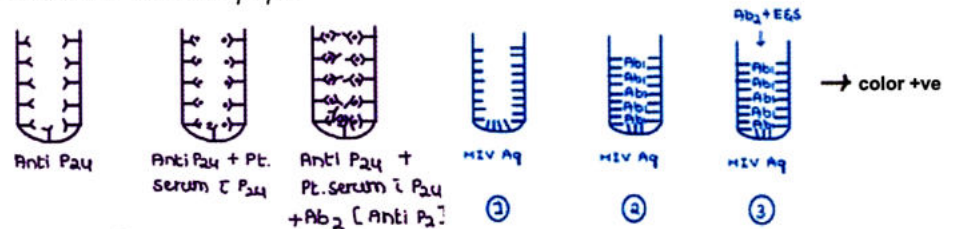
→ Malaria card test

ELISA → 3 Types

1. Indirect ELISA

2. Competitive ELISA

3. Sandwich ELISA



Indirect ELISA

→ A well tagged with Ag taken

→ Patient serum with Ab 1 added to it

Ab₁ Injected to → Goat Produces Ab₂ (Antibody against Ab₁)

→ Ab₂ + Enzyme + substrate added → If colour appears - ve

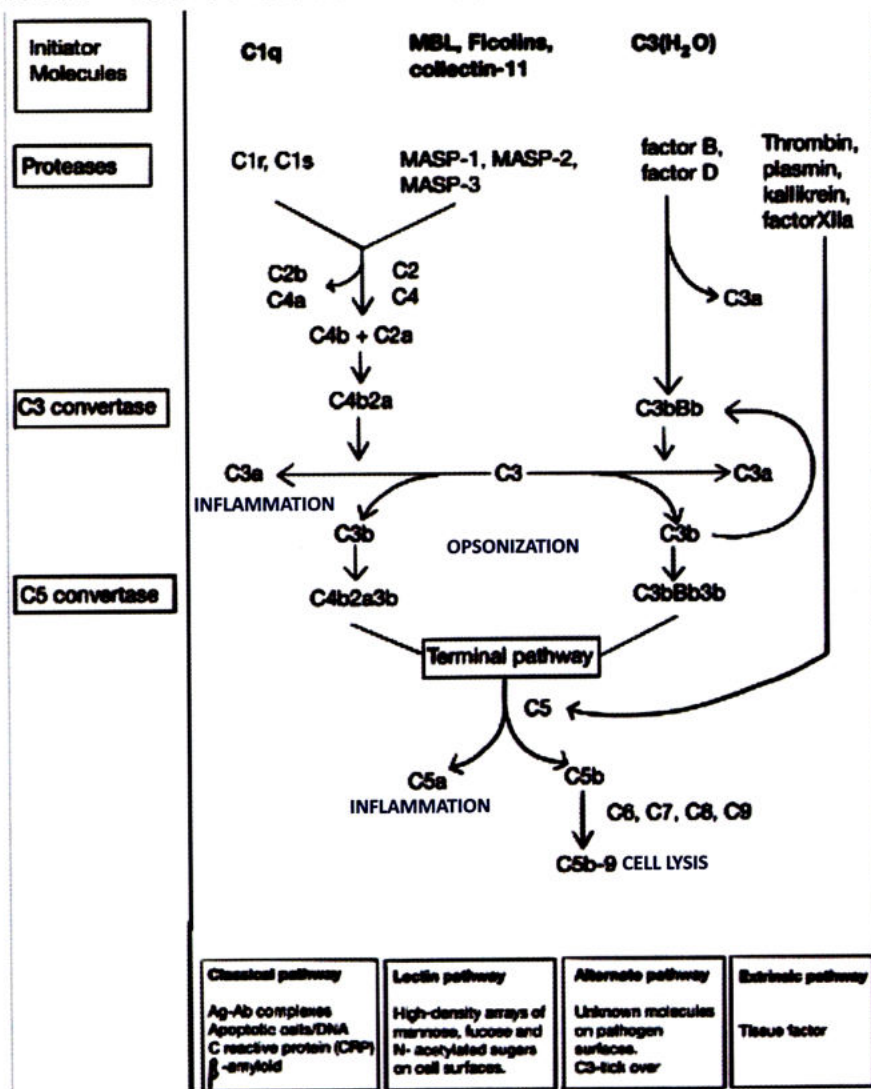
→ Can measure colour intensity by OD values (Optical density values)

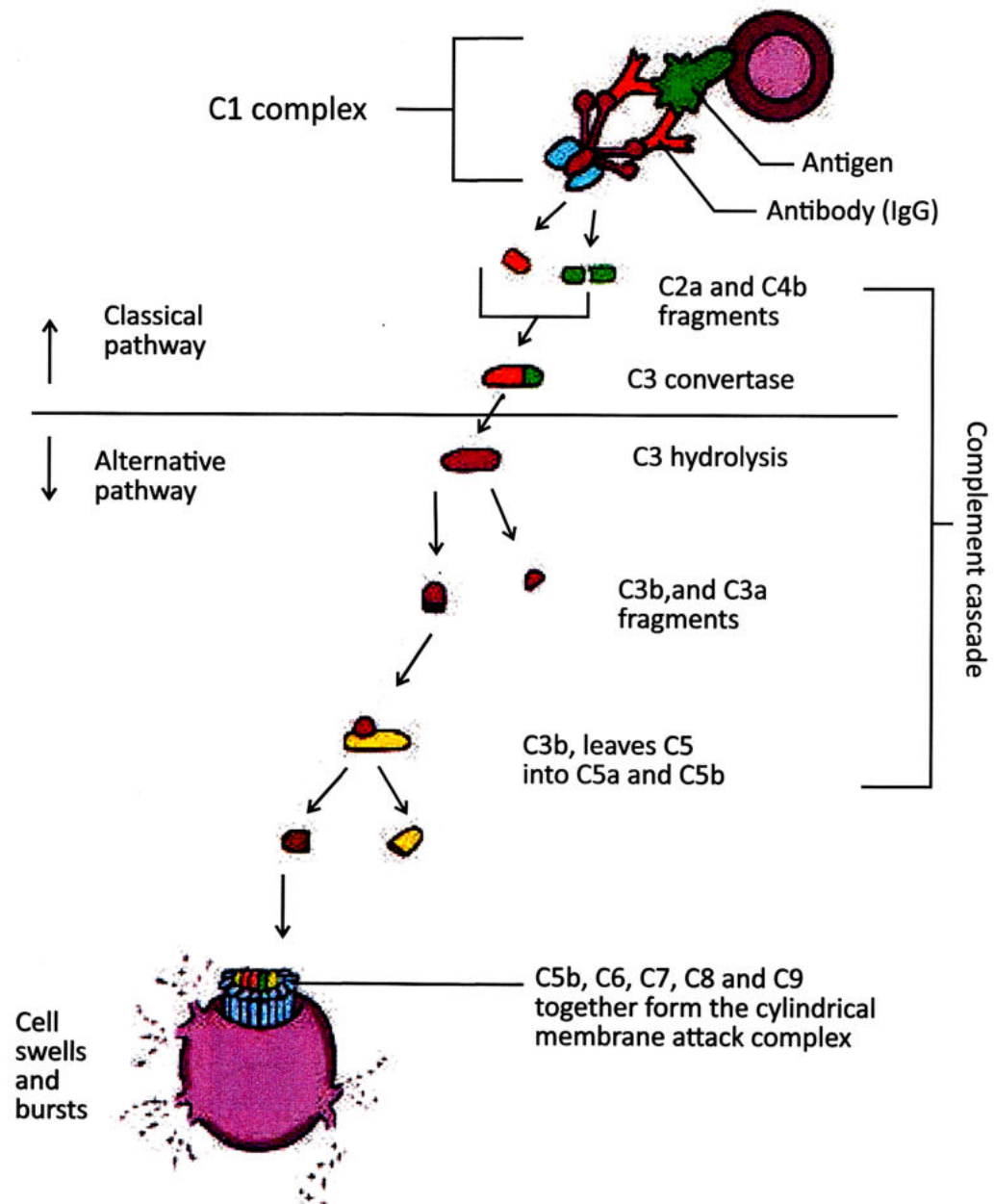
Fluorescent Testing

Direct → Antigen

Indirect → Antibody (Universal fluorophore is used)

Complement → are proteins ~ become inactivated at 56 C X 30 min

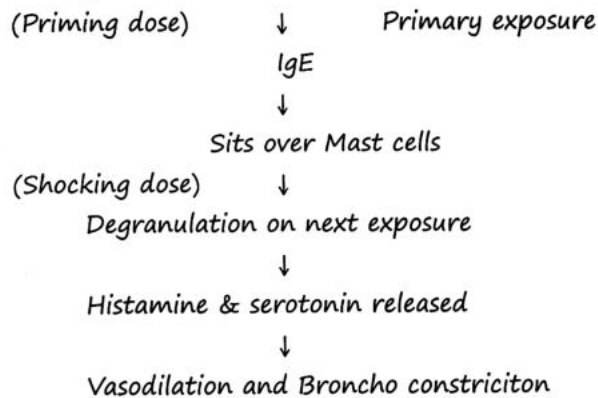




Hypersensitivity

- I → A → Anaphylactic Hypersensitivity
- II → C → Cytotoxic hypersensitivity
- III → I → Immune complex mediated hypersensitivity
- IV → D → Delayed hypersensitivity

TYPE-I → Allergen → Plasma cells



Eg → Asthma, Allergic Rhinitis

- Theobald smith phenomenon
- P - K reaction
- Casoni test
- Localised Anaphylaxis → Atopy
 - Food allergies
 - Pollen grains

Type II

- Cytotoxic
- Antigen + Antibody → Complement activation
 - ↓
 - Complement mediated phagocytosis (Opsonisation)
 - Complement mediated inflammation
- In Type II HS, antigen is the part of the cell (i.e. pre attached to cell surface) + Ab
 - ↓
 - Activation of complement
 - Complement mediated cytotoxicity
 - Complement mediated inflammation
 - Complement mediated phagocytosis.
 - Ab dependent cellular cytotoxicity
 - Ab dependent cellular dysfunction

E.g → Myasthenia gravis

- Grave's disease
- Autoimmune diseases
- Transfusion reaction
- Auto immune hemolytic anemia
- Good pasture syndrome
- Pemphigus vulgaris

Type 3 Hypersensitivity

Free antigen + Ab



Formation of Immune complexes



Complement activation

Eg → Arthus reaction

→ Serum sickness

→ PAN

→ SLE

→ Type II lepra reaction

Type IV HS



Sensitised 'T' lymphocytes



Release lymphokines



Granuloma

Eg → Tuberculin test

→ Lepromin test

→ Type I lepra reaction

→ Frei's test (LGV)

→ Contact dermatitis

Autoimmunity

1. Exposure of sequestered antigens

→ Sperms

→ Lens

2. Formation of Neo antigens due to

→ Biological exposure

→ Chemical exposure

3. Imbalance b/w cell mediated & humoral immunity

4. Cross reactivity of carbohydrate 'c' of streptococcus bacteria with cardiac valves and 'M' protein ↔ myocardium

5. Exposure of forbidden clones

Examples

1. Myasthenia gravis

2. Grave's disease

3. SLE

4. Hashimoto's thyroiditis

5. Rheumatoid arthritis → IgM against Fc portion IgG

B & T Cells

B Cells		T Cells	
Maturation ↓ Bone marrow	Both originate from bone marrow	Maturation ↓ Thymus	

T cells → 60 – 70%

B cells → 10 – 20 %

N K cells → 5 – 10%

T cells → CD1 to CD 8

B cells → CD 19 to CD 23

NK cells → CD16, CD56

Pan T cell marker → CD3

→ T cells + RBC = 'E' rosettes

→ B cells + RBC = EAC rosettes

T cells blast transformer

→ Phytohaemagglutinin & concanavalin

B cell blast transformer

→ Endo toxins

Antigen presenting cells

↓

Secrete IL – 1

↓

Act on T_H cell

↓

Produce IL – 2

↓

NK cells (non MHC restricted)

↓

Directly kill

→ Virus cells

→ Cancer cells

↓

LAK cells (Lymphokine activated killer cells)

IL 3 → GMCSF

IL 4 → Isotype switching to IgE

→ Plays a role in Type 1 Hypersensitivity

IL 5 → Isotype switch to IgA, IgE

Major Histocompatibility Complex

MHC → On chromosome 6p

MHC I → All Nucleated cells

MHC II → APC

MHC III → C₂, C₃, TNF, Heat shock proteins

Mechanisms

1. Defect in IL-2 receptor gamma gene
2. Adenosine deaminase (ADA) deficiency → Accumulation of ATP → Cause damage to cells
3. PNP – Purine Nucleoside phosphorylase deficiency
4. Reticular dysgenesis
5. Omenn syndrome
6. Bare lymphocyte Syndrome
7. Jak 3 deficiency (Janus Kinase)
8. Artemis deficiency

2. Ataxia Telangiectasia

3. Wiskott Aldrich Syndrome

- WASP gene defect
- R → Recurrent infections
- E → Eczema
- T → Thrombocytopenia
 - IgM ↓ es
 - IgG & IgA N
 - IgE – ↑ es

Disorder of Phagocytosis

1. Chronic Granulomatous Disease

- Deficiency of NADPH oxidase
- Catalase +ve organism infection common
- Nitroblue tetrazolium test → +ve

2. Chediak Higashi Syndrome

- LYST gene defect
- Defective phagolysosomal fusion
- ↓ In melanocytes

3. Job Syndrome (Hyper IgE Syndrome) → Frequent staphylococcal cold abscess are present.

4. Lazy Leukocyte Syndrome

Interferons

- Leucocytes → L – α
- Fibroblast → F – β
- T. Lymphocytes → T – γ
- Inhibits Viral protein Synthesis
- Species specific
- Used in Hep B & C
- Used in Multiple Sclerosis

Louis Pasteur

- Father of Microbiology
- Disapproved abiogenesis theory
- Coined germ theory
- Gave the concept of 3 Vaccines
 - C → Cholera A → Anthrax R → Rabies
- First Rabies vaccine administered to a boy named Joseph Meister
- Pasteurisation concept
- Liquid culture concept
- Some outlook regarding autoclave; hot air oven

**Robert Koch**

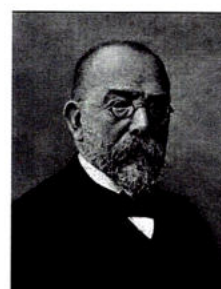
- Father of Modern microbiology
- Discovered TB and Cholera bacilli
- Gave solid media concept

Koch's Postulate

- Association
- Culture (Isolation) in pure culture media
- Inoculation into animal cause same disease
- Re-Isolation of organism from the animal
- Antigen enter body – Ab is produced

E.g → M.Tb causes TB, Therefore it will always be associated with Tuberculosis, can be isolated & if inoculated in an animal then will cause tuberculosis.

- Autopsy material in LJ → Reisolated
- Antigen → Ab

**Paul Ehrlich**

- Salvarsan / Magic bullet for syphilis
- Gave side chain theory for Ab production
- Acid fast staining

Joseph Lister → Father of antiseptic surgery

Antonie Van Leeuwenhoek

- Father of microscope
- Termed the microorganisms called Little animalcules

**Karry B Mullis** → Father of PCR

Characteristic	Viruses	Bacteria	Fungi	Protozoal Helminths
Cells	No	Yes	Yes	Yes
Approx. dia. (µm)	0.2 – 2	1–5	3–10 (Yeast)	15–25 (Trophozoite)
Nucleic Acid	DNA or RNA	DNA & RNA	DNA & RNA	DNA & RNA
Type of Nucleus	None	Prokaryotic	Eukaryotic	Eukaryotic
Ribosomes	Absent	70 s (30 s + 30s)	80 s (60s + 40s)	80s (60 s + 40 s)
Mitochondria	Absent	Absent	Present	Present
Outer surface	Capsid	Rigid wall Peptidoglycan	Rigid wall chitin	Flexible membrane
Motility	None	Some	None	Most
Replication	-	Binary fusion	Budding	Mitosis

Microscopy → Resolution → Ability to distinguish two points as separate

Resolution

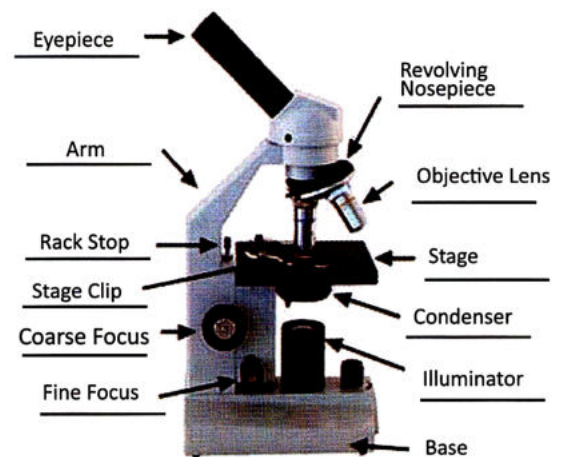
- Human eye → 0.2 mm
- Light microscope → 0.2 μ
- Electron microscope → 0.2 nm

Light Microscope

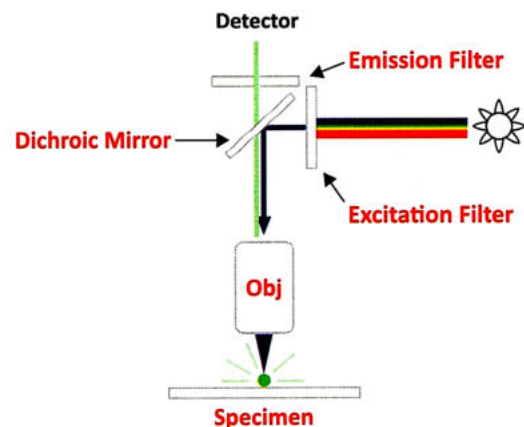
- Objective lenses (10 X, 40 X, 100 X)
- Eye Pieces (10 X)
- 100 X → Oil Immersion Lens
- Gram staining
- ZN staining
- 10 X, 40X → Demonstrate motility or Stool examination

Phase Contrast Microscopy is based upon

- Intensity of light
- Refractive indices
- Used to visualize motility and internal structures



Fluorescent Microscopy



- Short wavelength → Changed into long wavelengths

Fluorescent Dyes Are Used

- Auramine Rhodamine
- Acridine orange
- Calcofluor white

Electron Microscopy (Knoll & Ruska)

Electrons Are Used And Put Into Electromagnetic field

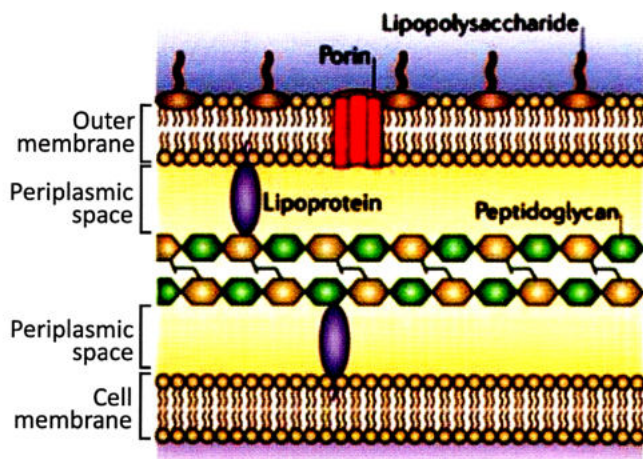
Prokaryotes Vs Eukaryotes

	Prokaryotes	Eukaryote
Nuclear membrane, nucleolus, RNP	Absent	Present
Cell division	Binary fission	Mitosis, meiosis
Chromosome	One, circular	Many, linear
Cell wall	NAM/NAG	Sterols (Also Mycoplasma)

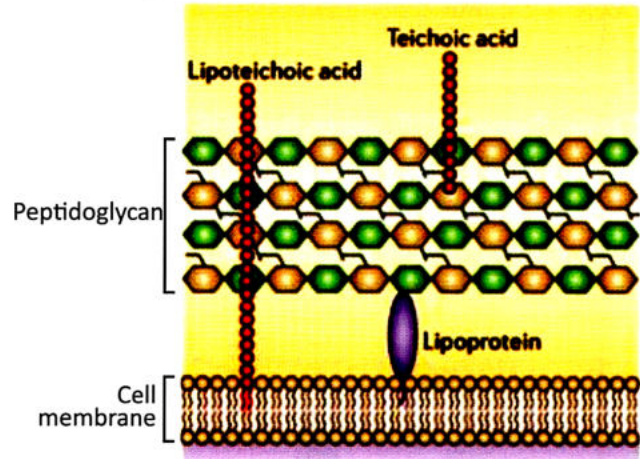
Membrane bound organelles	Absent	Present
Respiration	Mesosome	Mitochondria
Cytoskeleton	Absent	Present
Ribosome	70 s	80 s
Pinocytosis	Absent	Present
mRNA	No introns	Introns & Exons

Cell Wall

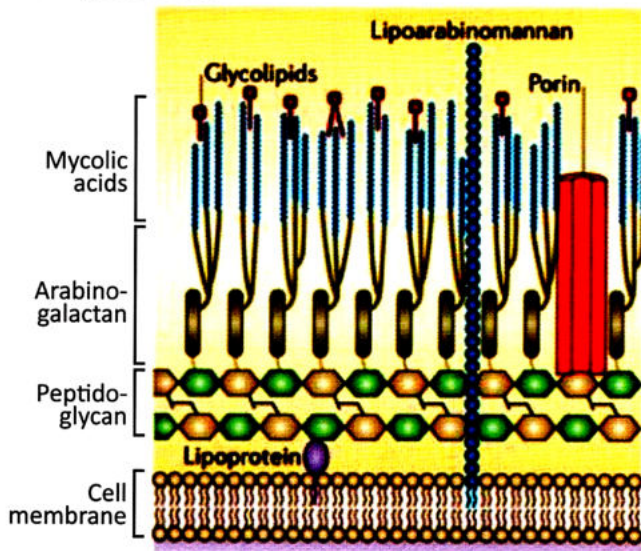
a Gram-negative bacteria



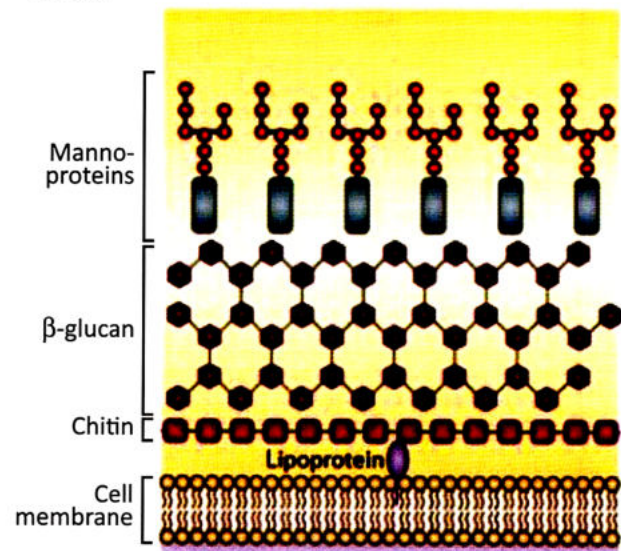
b Gram-positive bacteria



c Mycobacteria



d Fungi



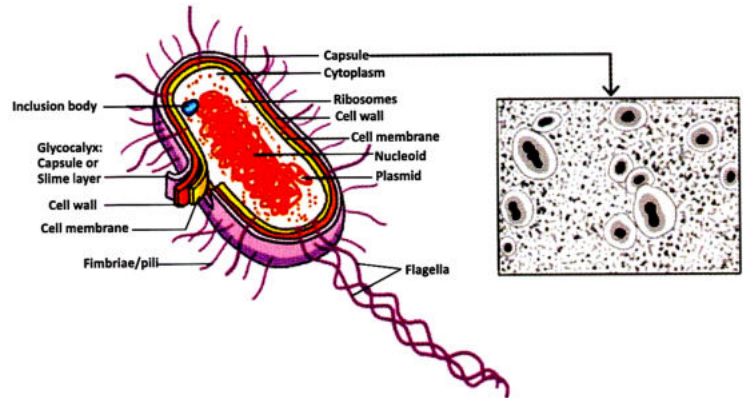
Difference Between Gram Positive And Gram Negative Cell Wall

Characters	Gram Positive Cell Wall	Gram Negative Cell Wall
Thickness	100 layer thick	1-2 layer thick
Lipid content	2-5% only	15-20%
Teichoic acid	Present	Absent
Variety of amino acid	Few	Several

Aromatic amino acid	Absent	Present
LPS (endotoxin)	Absent	Present
NAM (N acetylmuramic acid)	Each NAM molecule is attached with a tetra peptide side chain which are cross linked through a pentapeptide cross bridge	Tetrapeptide side chains are directly linked to each other No pentapeptide bridge.
NAG (N - acetylglucosamine)		

Cell Wall Demonstrated By

- Plasmolysis
- Micro dissection
- Reaction with specific antibody
- Mechanical rupture of cell
- Differential staining
- Electron microscopy

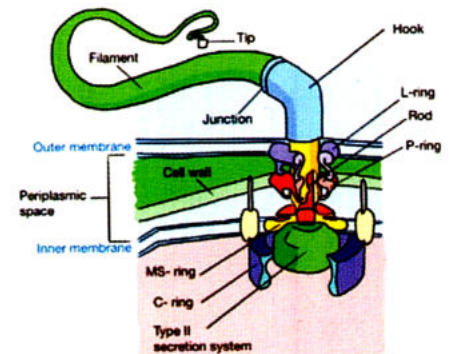


Capsule

- Avoid phagocytosis (Prevent opsonization)
- Adherence to surfaces
- Responsible for antibiotic resistance
- Most of the capsules are made up of polysaccharides – important Reaction shown is Quellung Reaction (Seen in Pneumococcus)
- Polypeptide capsule seen in – *Bacillus anthracis*
- Reaction is called Mc Fadyean's reaction.
- Negative staining by Indian ink staining for *Cryptococcus* capsule.

Flagella

- 0.02 μ thick
- 3 – 20 μ long
- Antigenic
- Used in Widal Test (Ab against 'H')



- Parts**
- Filament
 - Hook
 - Basal body
- } Attaches to cytoplasmic membrane by rings
- Tumbling motility → *Listeria*
 - Darting motility → *Vibrio cholera*
 - Swarming motility → *Proteus*
 - Falling leaf like → *Giardia lamblia*
 - Endo flagella → *Spirochaetes* (Arise from periplasmic space)

Fimbriae

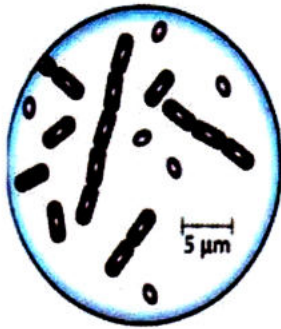
- 0.5 μ long; 10 nm
- Helps in Adherence to surfaces
- *E. coli* possesses 'P' fimbriae, attaching to uro epithelial cells → UTI

Spores

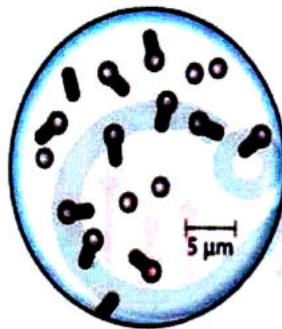
Centrally located	Drum stick app	Ovoid
-------------------	----------------	-------

- Drum stick appearance
- Subterminal spore
- Box car appearance

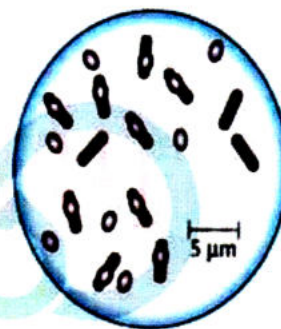
- *Cl. Tetani*
- Most of the clostridium
- *Bacillus anthracis*



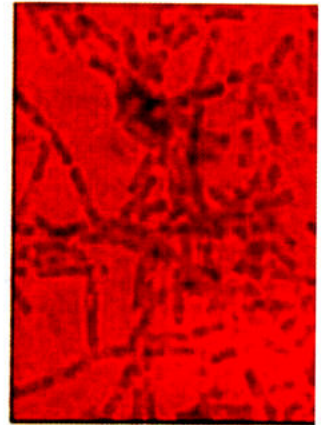
Spores ovoid; centrally located (*Bacillus cereus*)



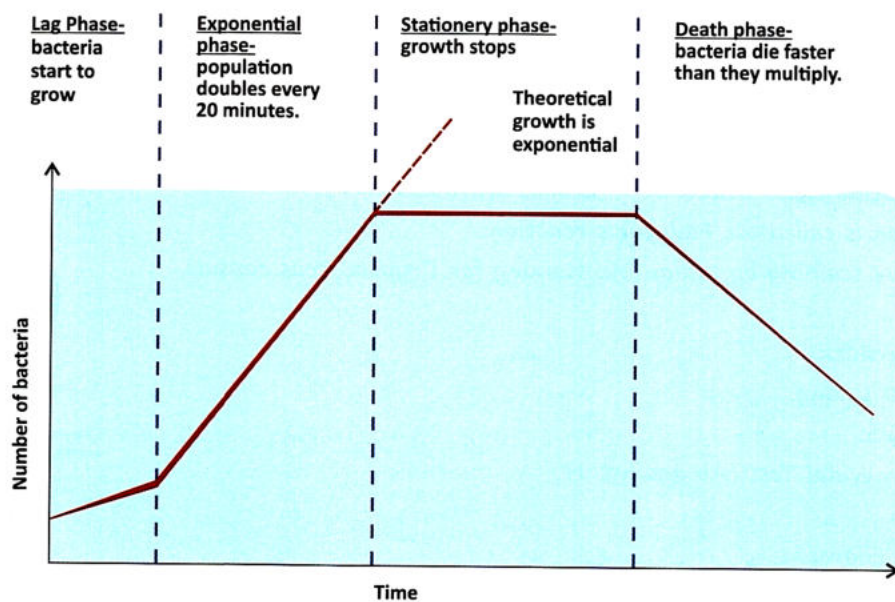
Spores spherical; terminally located (*Clostridium tetani*)



Spores ovoid; subterminally located (*Clostridium subterminale*)



Bacterial Growth Curve



Graphical Representation of a Bacterial Growth Curve

→ Sporulation happens in stationary phase

→ Decline phase – Involution forms

Protoplast (Gram Positive) → Without cell wall

Spheroplast (Gram Negative) → Without cell wall

L Forms

→ Cell wall free forms discovered by Lister Institute, London

→ Discovered in *Streptobacillus Moniliformis*

Sterilization & Disinfections

Sterilization → Making an article free of pathogens, non-pathogens, including spores.

Disinfection → kills/disinfect everything except spores.

Dry Heat Sterilization

- Incineration → Hospital waste
- Flaming → Inoculating loops
- Hot Air Oven → 160 C X 2 hrs
- Used For → Glass wares
- Grease, paraffin, dusting powder
- Cotton swabs

→ Surgical instruments

Control → Spores of *Bacillus subtilis* or spores of non-pathogenic (*Cl. tetani*).

Moist Heat Sterilization

- < 100 C → Inspissation
- Pasteurization
- = 100 C → Boiling
- Tyndallization
- > 100 C → Autoclave

Inspissation → 80 – 85C x 20 – 30 mins x 3 days

Done For

- LSS → Löffler's serum slope
- LJ media → Lowenstein Jensen Media (Because they can't be autoclaved)

Pasteurization

- Flash Method → 72 C for 15–20 secs & then temperature is brought down to 13C. It kills the *Coxiella Burnetti*
- Holder's → 63 C for 30 mins

Note → Flash method is better for pasteurization of milk.

Tyndallization

- At 100 C for 20 – 30 mins for 3 days
- Gelatin, sugar containing substances are tyndallised

Autoclave → At 121 C for 15 mins at 15 psi (per sq. Inch) pressure

Used For

- All culture media except LJM and LSS
- All surgical sutures except catgut sutures
- Surgical instruments
- Sputum samples before discard control
- spores of *Bacillus stearothermophilus*

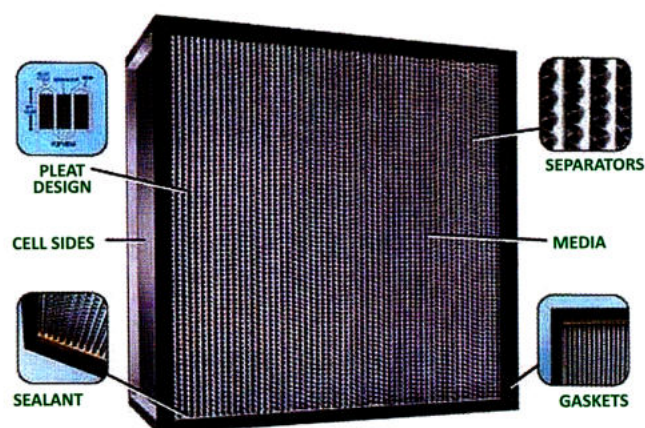
Filtration

- For Heat Sensitive Substances
- Filtration of serum / vaccines

Membrane Filters → (Pore size 0.22 μ)

HEPA Filters → High efficiency particulate air filters in (OTs) & biosafety cabinets.

→ > 0.3 μ size not allowed.



Ionising Radiation	Non Ionising Radiation
→ Gamma Rays & X Rays (High penetrating power)	→ <u>Infra Red & UV Light</u>
→ For Catgut sutures and pre packed syringes	→ For OT s & Biosafety cabinets
→ Method called cold sterilization	→ Method Called Host sterilization

Plasma Sterilization

- H_2O_2 gas is used
- From H_2O_2 Plasma radicals are produced
- Sterilization control → *B. Stearothermophilus*

Chemical Sterilization

- Phenol (Carbolic acid)
- Phenol is derived from coal tar
- Forms Cresol; Lysol
- Antiseptic → Dettol & Savlon (Chloro xylenol) (Chlorhexidine + Cetrimide)

Alcohols

1. Ethyl Alcohol → 60 – 70% concentration
 2. Isopropyl Alcohol → Handrubs & Sanitizer
- Stethoscope can be sterilized from isopropyl alcohol.

Note → Isopropyl alcohol is better than ethyl alcohol, as it is a better fat solvent.

Aldehyde

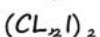
Formaldehyde

- Gaseous – OT fumigation
- Liquid – 10% – Formalin
- Anatomical & Pathological specimens are preserved in this.

Glutaraldehyde 2 % (Cidex)

- Bronchoscopes, Endoscopes, cystoscopes

Halogens



Chlorine → Sodium hypochlorite

- 1% Na Hypochlorite is used for – Blood spills (MOA- HOCl is formed i.e. strong oxidizing agent)

Iodine → As an antiseptic

- Tincture Iodine
- Betadine is a commonly used Iodophore

Ethylene Oxide

- Gaseous sterilant
- Cold Cycle → $37\text{ C} \pm 5\text{ C}$
- Warm Cycle → $54 \pm 5\text{ C}$
- It is an alkylating agent
- High penetrating power
- Explosive
- Pre packed syringes are better sterilized with alkylating agents than gamma radiation
- ICU equipments like respirators, Heart Lung machines
- Control – *B. Globigii*

Efficacy Of Disinfectant

1. Phenol Coefficient Test → Comparison between phenol and other disinfectants (Rideal Walker Coefficient)
2. Chick Martin Test → (Bacterial broth + organic waste (Faeces, yeast))
3. Kelsey Sykes Test → Serial dilutions of disinfectant- in use test commercially used in hospitals.

Spaulding Classification

- Non Critical Devices (Low Level Disinfection) → Comes in contact with intact skin (BP cuff, stethoscope)
- Semi Critical Devices (High level disinfection) → Comes in contact with mucous membranes
E.g. → Endoscopes, laryngoscopes
- Critical Devices (High Level Disinfection) → Which enter sterile sites
E.g. Surgical instruments, Implants, eye & dental equipment

Culture Media	→ Autoclave
Sharp Instruments	→ 5 % Cresol
Thermometer	→ Savlon

Bacterial Genetics

- Central dogma → DNA → RNA → Protein
 - Start Codon → AUG
 - Stop Codon → UAA, UAG, UGA
- ↓ ↓ ↓
 Ochre Amber Opal

Plasmid → Extrachromosomal DNA Substances

- Can be circular & double stranded DNA molecule that encodes the traits
- Not essential for survival

Types Of Plasmids

- F factor (Fertility factor)
- R factor (Resistance factor)
- Col factor

Plasmids Confer New Properties To Bacteria

- Resistance to antibiotics
- Induce production of toxins
- Plasmid when integrates with host chromosome called episome

Phenotypic Variations

- Salmonella Typhi is flagellated but when grown in phenol agar it produces – No flagella
- Inducible enzyme → Like β - Galactosidase

↓

Only produced by E. coli when grows in lactose containing media.

Genotypic Variations

- Mutation → Random, Undirected heritable variation
→ Caused by change in the nucleotide sequence of DNA of cell

→ Frequency - 10^{-2} to 10^{-10} per bacterium per division

Types Of Mutations

- Point Mutation**
- Base pair substitution
 - Frame shift mutation
- Multisite Mutation**
- Addition
 - Deletion
 - Duplication
 - Inversion

Base Pair Substitution

- Transition**
- Purine is replaced by purine
 - Pyrimidine is replaced by pyrimidine
- Transversion**
- Purine by pyrimidine & vice versa

Frame Shift Mutation

- DNA Replication → One or few base pairs inserted into/ deleted from DNA
Shift the normal reading frame

↓
Formation of altered codons

↓
Code for Different amino acid (Missense Mutation)

Non Sense Mutation → Premature protein synthesis termination by stop codons.

- Tests For Mutation**
- Fluctuation test
 - Replica plating
 - Amie's test

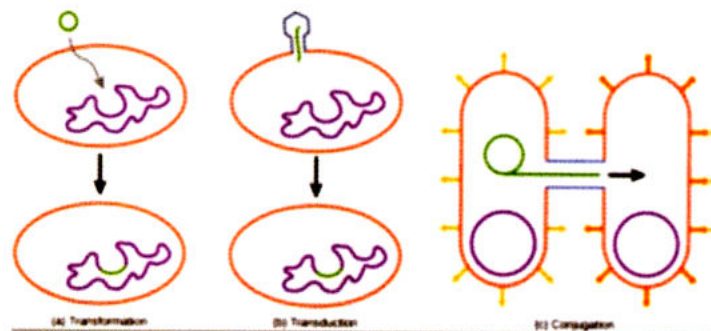
Mechanisms Of A Resistance Transfer

- Conjugation
- Transformation
- Transduction

Transduction

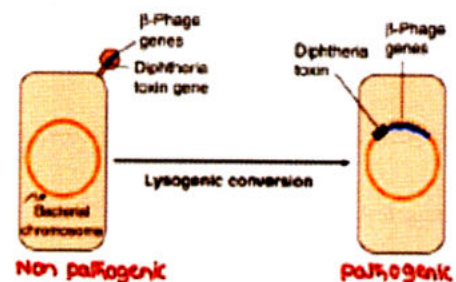
Lysogeny

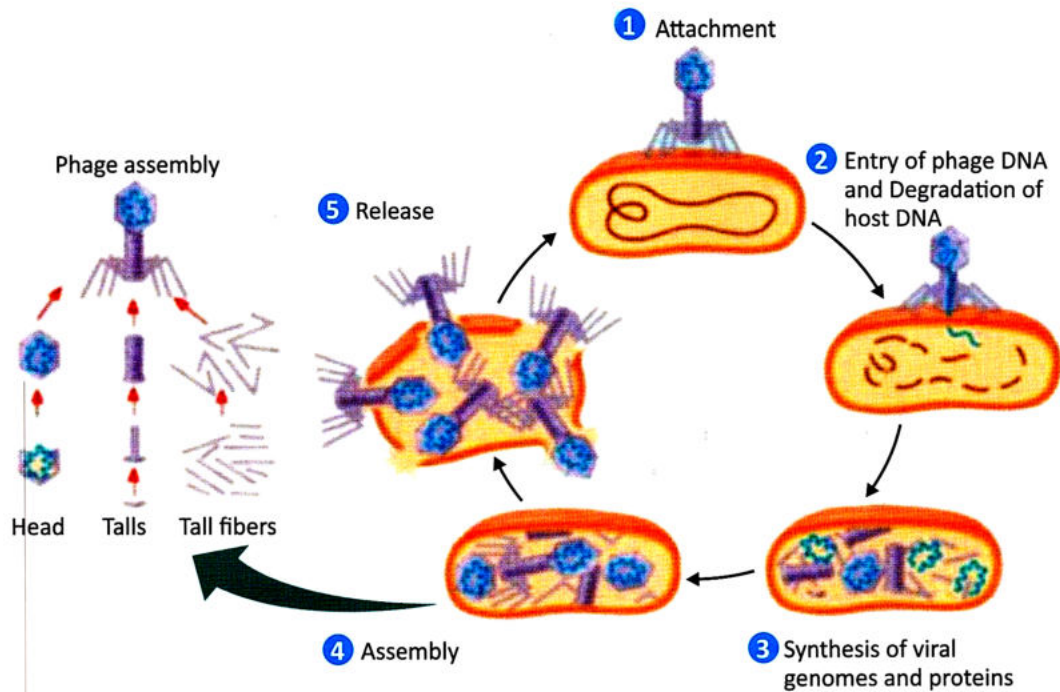
- Prophage / Recombinant
- Bacteriophage mediated transfer



Lytic → Cycle

E.g. → Non-pathogenic CD changes to pathogenic CD (*Corynebacterium Diphtheriae*) by β phage tox gene.





Transformation → Griffith experimented on pneumococcus

Pneumococcus Strains

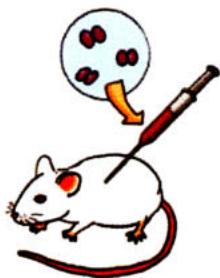
(R) Non capsulated

(S) Capsulated (Virulence in Capsule)

→ Heat Killed Capsulated Pneumococcus + Live Non Capsulated Pneumococcus Injected into mice → Mice died

→ Inside the dead mice live capsulated pneumococcus present, this occurs via agency of free DNA.

1 Living nonencapsulated bacteria injected into mouse.

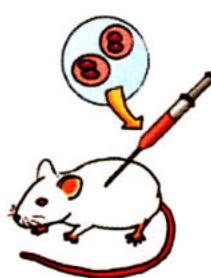


2 Mouse remained healthy.

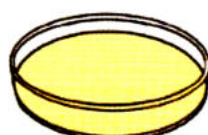


3 A few colonies of non-encapsulated bacteria were isolated from mouse; phagocytes destroyed non-encapsulated bacteria.

1 Heat-killed encapsulated bacteria injected into mouse.

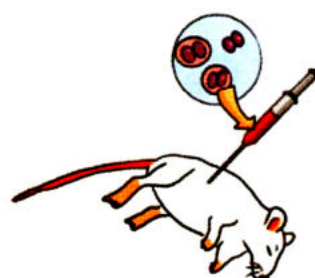


2 Mouse remained healthy.

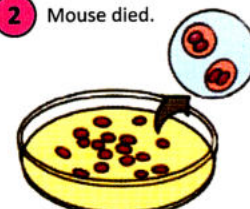


3 No colonies were isolated from mouse.

1 Living non-encapsulated and heat-killed encapsulated bacteria injected into mouse.



2 Mouse died.



3 Colonies of encapsulated bacteria were isolated from dead mouse.

